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61

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CONTENTS.

1. **The Toxicology of Nickel Carbonyl.** Pt. II. By H. W. Armit. (*Journal of Hygiene,* Vol. VIII., p. 565, 1908.)
2. **Bacillus Dysenteriae of Flexner in Relation to Asylum Dysentery.** By H. T. S. Aveline, A. E. Boycott, and W. F. Macdonald. (*Journal of Hygiene,* Vol. VIII, 1908.)
3. **The Prevention of Compressed-Air Illness.** By A. E. Boycott, G. C. C. Damant and J. S. Haldane. (*Journal of Hygiene,* Vol. VIII., 1908.)
4. **The Effects of Low Atmospheric Pressures on Respiration.** By A. E. Boycott and J. S. Haldane. (*Journal of Physiology.,* Vol. XXXVII., 1908, p. 355.)
5. **On the Origin of Endogenous Uric Acid** By E. P. Cathcart, E. L. Kennaway and J. B. Leathes. (*Quarterly Journal of Medicine,* 1908.)
6. **The Principles involved in the Standardisation of Disinfectants and the Influence of Organic Matter upon Germicidal Value.** By Harriette Chick and C. J. Martin. (*Journal of Hygiene,* Vol. VIII., 1908, p. 654.)
7. **A Comparison of the Power of a Germicide Emulsified or Dissolved, with an Interpretation of the Superiority of the Emulsified Form.** By Harriette Chick and C. J. Martin. (*Journal of Hygiene,* Vol. VIII.. 1908, p. 698.)
8. **Observations Indicating that the recent Outbreak of Diphtheria in the Wood-Pigeon (Columba Palumbus) is caused by a "Filter-Passer."** By G. Dean and W. E. Marshall. (*Journal of Pathology and Bacteriology,* Vol 13, 1908.)
9. **Some Experiments on Immunity against Vaccinia in Animals** By B. Alan Green. (*Journal of Hygiene,* Vol. VIII., 1908.)
10. **The Influence of Temperature and some other Physical Conditions on Calf Vaccine.** By Alan B. Green (*Journal of Hygiene,* Vol. VIII., 1908.)
11. **An Outbreak of Cow-Pox.** By Alan B. Green. (*Lancet,* Vol. 2, 1908.)

12. **The Alcoholic Ferment of Yeast-Juice.** Part III. **The Function of Phosphates in the Fermentation of Glucose by Yeast-Juice.** By A. HARDEN and W. J. YOUNG. (*Proc. Royal Society,* B. Vol. 80, 1908.)

13. **The Effects of Muscular Work upon the Excretion of Endogenous Purines.** By E. L. KENNAWAY. (*Journal of Physiology,* Vol. XXXVIII., 1908.)

14. **A Preliminary Note on the Examination of the Fat in the Liver in Health and Disease.** By E. L. KENNAWAY and J. B. LEATHES. (*Proc. Royal Society of Medicine,* February, 1909.)

15. **On the Presence of Hæmolytic Factors in Milk.** By JANET E. LANE-CLAYPON. (*Journal of Pathology and Bacteriology,* Vol. 13, 1908.)

16. **On Changes in the amount of Higher Fatty Acids to be obtained from the Liver after Removal from the Body.** By J. B. LEATHES. (*Archiv für Experimentalle Pathologie und Pharmakologie. Suppl. Band.* "*Schmiedeberg Festschrift,*" 1908.)

17. **A Lecture on the Functions of the Liver in Relation to Metabolism of Fats.** By J. B. LEATHES. (*Lancet,* Vol. I., 1909.)

18. **On the Desaturation of Fatty Acids in the Liver.** By J. B. LEATHES and L. MEYER-WEDELL. (*Proc. Physiological Society,* February, 1909.)

19. **On the Influence of Temperature on Phagocytosis.** By J. C. G. LEDINGHAM. (*Proc. Royal Society,* Vol. B, 80, 1908.)

20. **The Typhoid-Carrier Problem, with some Experiments on Immunity in Carriers.** By J. C. G. LEDINGHAM. (*British Medical Journal,* Vol. 2, 1908.)

21. **Bile Salt Media and their Advantages in some Bacteriological Examinations.** By A. T. MACCONKEY. (*Journal of Hygiene,* Vol. VIII., 1908.)

22. **On the Relationship between Bacillus Pestis and Bacillus Pseudo-Tuberculosis Rodentium (Pfeiffer).** By A. T. MACCONKEY. (*Journal of Hygiene,* Vol. VIII., 1908.)

23. **On a Hæmogregarine from the Blood of a Himalayan Lizard (Agama Tuberculata).** By E. A. MINCHIN. (*Proc. of the Zoological Society, London,* 1907, p. 1098.)

24. **Note on the Polymorphism or Trypanosoma Gambiense.** By E. A. MINCHIN. (*Parasitology,* Vol. I., 1908.)

25. **The Bacteriology of Summer Diarrhœa.** By H. DE R. MORGAN and J. C. G. LEDINGHAM. (*Proc. Royal Society of Medicine, Epidemiological Section,* March, 1909.)

V

26. **Further Results of the Experimental Treatment of Trypanosomiasis: being a Progress Report to a Committee of the Royal Society.** By H. G. Plimmer and H. R. Bateman. (*Proc. Royal Society*, B. Vol. 80, 1908.)

27. **Alveolar Air on Monte Rosa.** By R. Ogier Ward. (*Journal of Physiology*, Vol. XXXVII., 1908, p. 378.)

28. **A Note on the Variation of the Rate of Disinfection with Change in the Concentration of the Disinfectant.** By Herbert E. Watson. (*Journal of Hygiene*, Vol. VIII, 1908.)

29. **On the Occurrence of Nuclear Dimorphism in a Halteridium Parasitic in the Chaffinch, and the probable connection of this Parasite with a Trypanosome.** By H. M. Woodcock. (*Quarterly Journal of Microscopic Science*, Vol. 53, Part 2, 1909, p. 339.)

XI

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THE TOXICOLOGY OF NICKEL CARBONYL.

Part II.

Plates VII—IX, and Four Figures.

By H. W. ARMIT.

(*From the Lister Institute of Preventive Medicine.*)

I. Introduction.

Nickel responsible for the symptoms of Nickel Carbonyl poisoning.

In Part I[1] it was shown that the toxic symptoms after nickel carbonyl inhalation are not due to either the nickel carbonyl as such or to the carbon monoxide which is given off when the compound is exposed to air and moisture at body temperature. It was therefore concluded that they are occasioned by the absorption of the nickel set free and that nickel carbonyl poisoning is a particular case of nickel poisoning.

The fact that the nickel is deposited over the immense surface of the lungs in a condition which is especially favourable for absorption renders a study of the toxicology of this compound of great interest.

The results of this second part of the investigation will be presented in the following order:

1. Further evidence that the nickel is responsible for the symptoms, by comparison of the course of poisoning and of the changes induced by the inhalation of nickel carbonyl vapour with the course of poisoning and changes induced by the administration of other nickel compounds.
2. An attempt to trace the nickel through the organism.
3. A comparison of the toxic effects of nickel, iron and cobalt.

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[1] See *Journ. of Hygiene*, vii. 4, pp. 525—551. 1907.

II. COURSE OF POISONING IN ANIMALS.

(i) *Methods employed.*

The experiments with nickel and iron carbonyl were, save for a few of the earlier ones, in which another method which did not prove satisfactory was employed, carried out as follows:—the liquid carbonyl was placed in a graduated burette of 1 c.c. capacity (see Plate IX *e*) provided with an ordinary stopcock above and a three-way stopcock below. The lower end of the burette was passed through a rubber cork, fitted into a glass receiver (*f*) which contained a little cotton wool to catch the carbonyl. A given quantity of the carbonyl was run out of the burette by turning on the three-way stopcock. The tap having been reversed, a small quantity of carbon dioxide was allowed to pass through the receiver containing the nickel carbonyl into a large gasholder (A), the capacity of which was approximately 240 litres so as to evaporate the carbonyl in the receiver. A little air was blown into the gasholder before the carbon dioxide was turned on, to prevent the carbonyl condensing in the latter. While the carbon dioxide was blowing all the carbonyl over, air was introduced into the gasholder through the second tap (*d*). The quantity of carbonyl being known (1 c.c. of liquid nickel carbonyl yields 181·1 c.cm. of vapour at 0° C. and 760 mm. Hg., and 1 c.cm. of iron carbonyl yields 167·39 c.cm. of vapour) and the capacity of the gasholder having been determined and recorded on a scale (*c*) attached to the upright, the volume percentage of the carbonyl in the air could be accurately measured. After all the fluid had been evaporated, the taps of the gasholder were closed and the heavy weights exchanged for one of two kilograms. The carbonyl burette was removed and the chamber (B) was connected to the gasholder by applying the tube *g* to the tap *b*. The chamber was an air-tight box, made of mahogany and glass with rubber stoppings adjusted to the windows and lid. From the outlet (*h*) a tube carried off the vapour and air to a Bunsen burner, where all the carbonyl was dissociated. A screw clamp applied to this tube regulated the flow of the vapour mixture passing through the chamber. The rate was determined by timing the descent of the level of the top of the gasholder against the scale (*c*). It was found that a satisfactory rate for the purpose was three litres per minute.

The poison chamber contained air at the beginning of each experiment, and this air was gradually replaced by the vapour mixture in the gasholder. In order to determine the rate of replacement, test experiments were conducted with oxygen (in which Dr Boycott, of Guy's Hospital, kindly assisted me) and the gas issuing from the outlet was analysed at short intervals. The first series of determinations were conducted with an inanimate object in the chamber in the place of the rabbit. The second series was conducted with a living rabbit, and it was found that the mechanical action of the respiration, etc. on the mixing of the gases was appreciable. With the rate of flow employed the quantity of oxygen absorbed by the rabbit may be neglected. It was therefore considered better to utilise the results of the second series alone, and from the figures obtained a curve of the rate of displacement was interpolated, the ordinates representing the percentage of vapour mixture which has replaced the air in the chamber and the abscissa representing the time in minutes. The figure lying above the curve has been plotted as a rectangular figure, and from this it appears that a deduction of 14½ minutes would be a necessary correction in taking the time during which the animal was in the poison chamber as indicating the opportunities for intake of nickel carbonyl vapour. The curve and rectangular figure is given in Fig. 1).

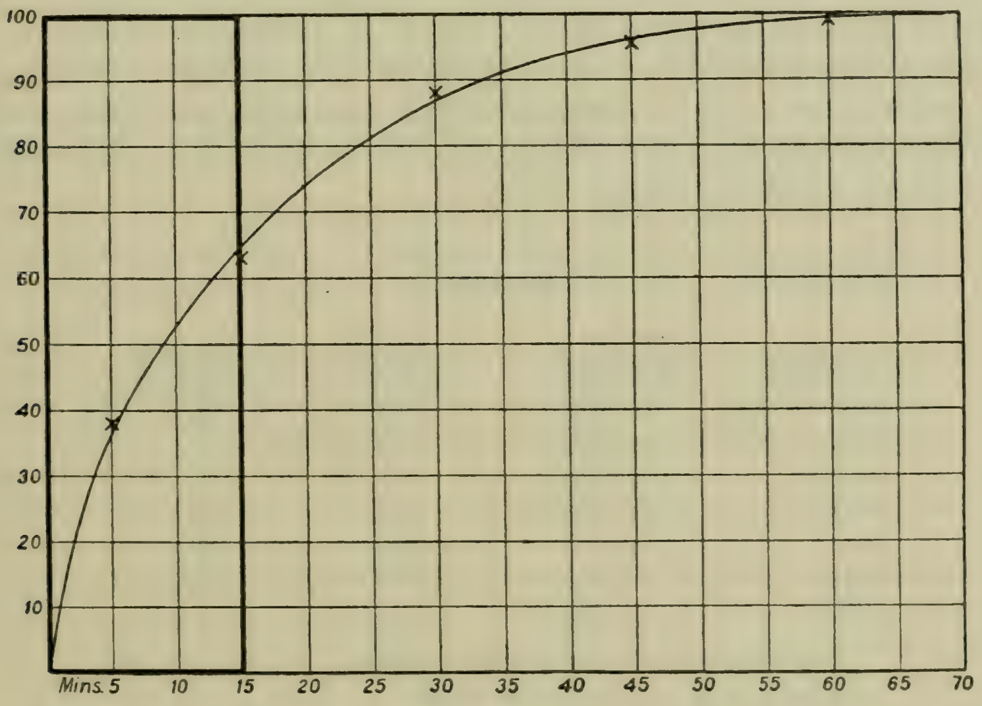

Fig. 1. Curve showing Rate of Displacement in chamber.

It must however be pointed out that nickel carbonyl would not behave like a stable gas, such as carbon monoxide, on account of its ready dissociability in the presence of air and moisture at the temperature of the animal body (see Part I) and the results obtained by means of this curve are only approximate.

(ii) *Concentration of vapour and length of exposure.*

A large number of the experiments with nickel carbonyl are available for the purpose of determining how much of the vapour has to be inhaled to produce death. Naturally some individual idiosyncrasy played a considerable rôle in the inhalation experiments. One hundred and twenty-two rabbits were exposed to atmospheres containing varying volume percentages of nickel carbonyl vapour in air for varying periods. After a few preliminary experiments, the time factor was kept constant in a long series and the concentration was varied. When inhaled for 65 minutes, calculated from the time of establishing a connection between the gasholder and the poison chamber, 0·018 to 0·0188 volume per cent. of nickel carbonyl in air killed 64 out of 77 rabbits, i.e. 83·11 %. It would thus appear that, after making the 14½ minutes allowance as suggested above, 0·018 volume per cent. was capable of killing when inhaled for 50½ minutes. Variations of the time and concentration factors led to the following results:

TABLE I.

	Concentration of Ni (CO)	Ratio of concentration to 1st series	Length of whole experiment	Time after deduction of 14½ minutes	Result
1.	0·018 vol. %	100 : 100	65	50·5	†
2.	0·04 ,,	222 : 100	40	25·5	R
3.	0·075 ,,	416 : 100	31	16·5	R
4.	0·016 ,,	88 : 100	72	57·5	†
*5.	0·015 ,,	83 : 100	108	93·5	†
6.	0·0136 ,,	72 : 100	79	64·5	R
7.	0·0134 ,,	74 : 100	82	67·5	†

* This was one single rabbit and the time was unnecessarily long.

† signifies died. R signifies recovered.

From these figures it will be seen that the concentration of vapour necessary to kill an animal was in inverse proportion to the time the vapour was inhaled.

Cats withstand the effect of the vapour better than rabbits. After inhaling the carbonyl for 90 minutes (i.e. 75·5 minutes after making the deduction) 25 out of 30 animals, i.e. 83·3 %, died when 0·04 volume per cent. of the vapour in the air was used.

Dogs die after inhaling air containing 0·036 volume per cent. of nickel carbonyl for 90 minutes (i.e. 75·5 minutes after the deduction is made).

(iii) *Course of poisoning.*

The effect of the inhalation of the vapour of nickel carbonyl by rabbits may be illustrated by the following account of a typical case.

Rabbit 12 (second series). Weight 1935 grams. Temp. 39·4° C.

0·2 c.cm. of liquid nickel carbonyl was evaporated into the gasholder and to the total quantity of air made up to 192·75 litres, i.e. 0·0188 volume per cent.

The vapour mixture was allowed to pass through the chamber at 10.20 a.m. The respiratory rate was increased after 10 minutes to 132 in the minute, probably on account of apprehension, but soon fell again to 90. After 25 minutes, some uneasy movements were performed. After 34 minutes, the respiration was 176 and the rabbit became extremely restless. The respiration increased in rate steadily from this time and dyspnoea was seen. The restlessness passed off. At the end of one hour, the respiratory rate was 180, the rabbit passed urine and faeces but remained quiet. The visible vessels in the ears did not show any change of colour, but the lips and tongue were cyanotic.

The rabbit was taken out at 11.25, i.e. after having been in the chamber for 65 minutes.

At 11.30, the respiratory rate was 180, the dyspnoea had disappeared and the animal appeared comfortable. The temperature taken at 2.45 p.m. was 38·8° C. and the respiratory rate 120. During the rest of the day the rabbit seemed to be but little affected.

On the following morning the weight had decreased to 1712 grams, the temperature was 38·0° C., the respiratory rate was 196; there was dyspnoea, slight cyanosis of the lips and tongue, and on ausculting the chest, a prolongation, roughening and loudening of the inspiration was heard over the inter-scapular space and also to a less degree in the axillae. This gave the impression of reversed bronchial breathing. The expiratory sounds were unaltered and no râles were heard. The rabbit was taking very little food, but on being allowed out of its cage, was quite lively. During the afternoon, the dyspnoea increased and on the following morning (i.e. 48 hours after the inhalation) it seemed very ill. The weight was 1673 grams, the temperature 38·3° C., and the respiratory rate 204. There was much dyspnoea and cyanosis. It refused food altogether. The physical signs had increased considerably. 24 hours later a distinct improvement was seen, the respiratory rate was 156 and the dyspnoea was not so marked, while the temperature was 39·4° C. The weight however was 1607 grams. At the end of 96 hours, the weight had dropped to 1570 grams, i.e. a loss of nearly 19 °/₀ of its original weight. The respiratory rate was 132, there was urgent dyspnoea again and extreme cyanosis. The temperature had fallen to 34·5° C., and the animal was apathetic and ill. The physical signs during the last 24 hours revealed consolidation of both lungs, but there was no absolute dulness to percussion. The character of the breath sounds was more tubular than at first, and there were a few râles to be heard. It died at 1 p.m. i.e. 98½ hours after poisoning.

The following chart shows the variations of temperature and respiratory rate.

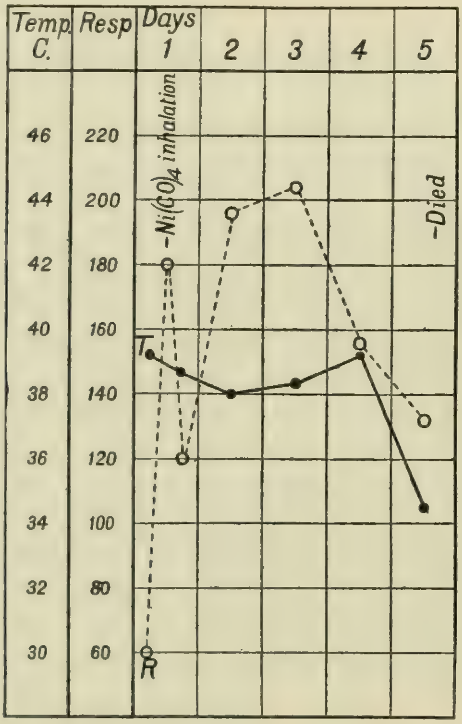

Fig. 2. Chart of Rabbit 12.

(iv) *Variations.*

In other cases, some variations of the symptoms were noted. In cases in which the death took place late, the respiratory rate became very slow but the dyspnoea increased up to the end. In one instance a rabbit died after 204 hours. During the last 12 hours of life, the respiratory rate was only 24 in the minute. When the inhalation was prolonged for a considerable time, and when strong concentrations of vapour were used, the lung symptoms became more intense.

The heart was rarely affected, the rate however usually failing to keep pace with the respiratory rate. Reduplication of the first sound at the apex was heard at times. The peripheral vessels were frequently contracted from the third day onward.

As a rule, symptoms referable to disturbance of the central nervous system were not well marked. It is difficult to decide whether the dyspnoea and cyanosis were entirely due to the changes in the lungs. In a few cases, there was paresis of the hind legs. The muscular tone was diminished or lost, the muscle most frequently affected being the sphincter ani. From the second day onwards there was loss of sexual desire. Urine was retained for some hours before death in a number of cases. Final convulsions were frequently seen. The animals nearly always assumed an orthotonic position before death.

The temperature curve was of the type shown above in the majority of the rabbits. Modifications however were seen, including the following:

(*a*) gradual fall after 24 to 36 hours,
(*b*) sudden fall at 24 hours to 35° C. or lower,
(*c*) fall within 18 hours, with subsequent rise and later fall.

Death took place between 60 and 90 hours after poisoning in a large number of experiments. The average for the 64 rabbits poisoned by inhaling 0·018 volume per cent. of nickel carbonyl for 50½ minutes was 69·2 hours.

The variations noted included:

(*a*)	Immediately after poisoning	(1 in 122).
(*b*)	Between 23 and 36 hours	(11 in 122).
(*c*)	Between 100 and 150 hours	(4 in 122).
(*d*)	Over 200 hours	(2 in 122).

Some rabbits appeared to be very resistant toward the effect of the vapour and this resistance appears to vary at different times in the same animal. A previous non-fatal poisoning dose appears to render the rabbit slightly less susceptible to the vapour, provided that sufficient time is allowed to elapse for the obvious lesions to be repaired. Independent diseases, such as tuberculosis, render rabbits more susceptible to nickel carbonyl.

In Part I of this paper, reference was made to the examination of the blood for carbon monoxide. The maximum amount of carbon monoxide found in any one case was equivalent to a saturation of not more than 5 % of the haemoglobin.

A number of rabbits were poisoned with quantities of nickel carbonyl just sufficient to produce death, and the blood was examined daily at the same time. It was found necessary first to control the blood counts for several days, prior to the poisoning, since some rabbits exhibit considerable variations. Only those rabbits which gave steady counts were used. The number of red corpuscles remained unaltered until the 4th or 5th day, when a reduction to about ⅘ of the original number was observed. The haemoglobin value fell at about the same time, but the lowest values were only about 85 % of the full values.

During the first three days, the leucocytes tended to decrease in number but never reached below the lower limit of a normal count. The slight drop was followed by a recovery to the former level. Just before death a distinct leucocytosis was seen. The polymorphonuclear elements were the only leucocytes increased in number. A curve is appended to illustrate the type of the changes (Fig. 3).

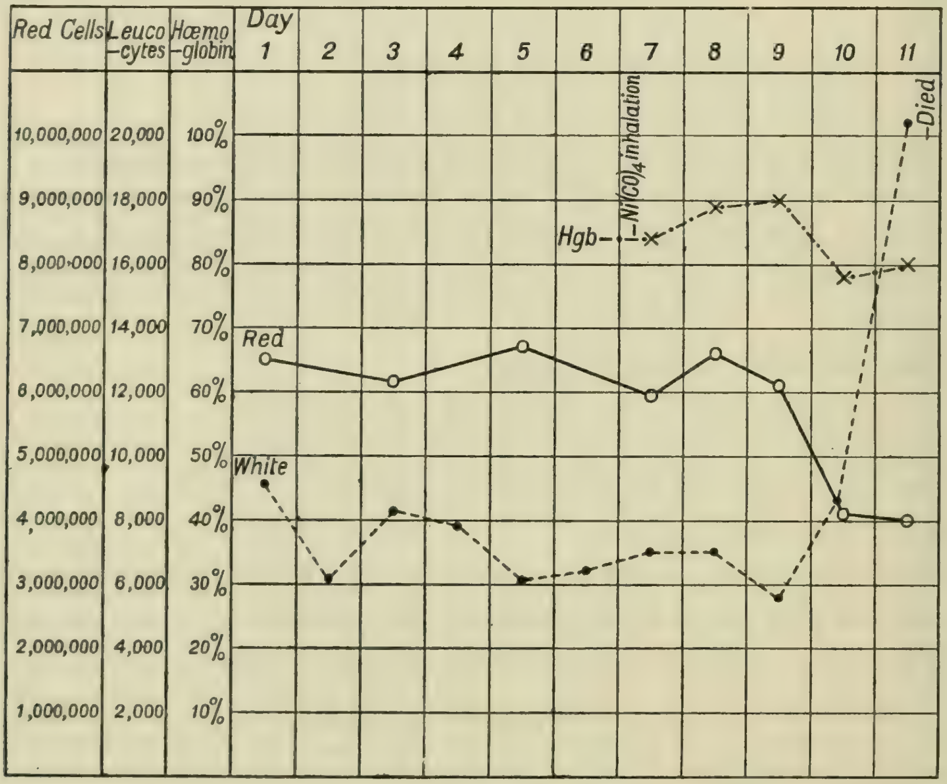

Fig. 3.

(v) *Poisoning in cats.*

The following is an example of the typical course of nickel carbonyl poisoning in cats:

Cat 11. Weight 3340 grams.

0·42 c.cm. of liquid nickel carbonyl was evaporated into the gas holder and the total quantity of air was made up to 189·8 litres, i.e. 0·04 volume per cent.

During the inhalation there was preliminary increase of the respiratory rate, followed by ordinary breathing, and after about 25 minutes, rapid and difficult breathing was evidenced. Toward the end of the inhalation, faeces were passed and the cat vomited. There was salivation. On being taken out at the end of 90 minutes, it was obviously very giddy, dyspnoeic and somewhat cyanotic. At 4 p.m. the temperature was 39·4° C., the respiration was 42 in the minute and the heart was beating 180 in the minute. It was still distinctly affected from the inhalation but the dyspnoea was markedly less than it was when the cat was taken out of the poisoning chamber. On the following day the weight was 3170 grams, the temperature 38·5° C., the respiration 66 and the heart beat 156. There was some dyspnoea and the cat was dull and apathetic. The inspiratory sounds were harsh and loud all over the chest, but there were no added sounds, and no impairment of resonance. The dyspnoea increased during the day, and on the following morning was very marked. The weight had then dropped to 3118 grams, the temperature was 36·8° C., the respiratory rate 114 and the heart beat 102. The physical signs all over both lungs were typical. On the next day the weight still further decreased to 3010 grams, the temperature was 35·2° C., the respiration 78 and the heart beat 132. The reflexes were distinctly increased, the pupils were dilated but reacted to light, and there was marked cyanosis. Over the right lung, behind, there was impairment of resonance and the expiratory murmur was rough and prolonged in this situation. Otherwise the signs were unaltered. During the following days the weight decreased about 100 grams a day, while the respiratory rate decreased to 54, 30, 24, 22 and 23 on the successive days. The heart rate remained about 144 during the latter part of the illness. The temperature rose on the 5th day to 37° C., fell slightly on the 6th but rose again on the 7th day to 37·4° C. After this it fell to 33° C. and 31° C. before death. A curve showing the respiration, pulse rate and temperature is appended.

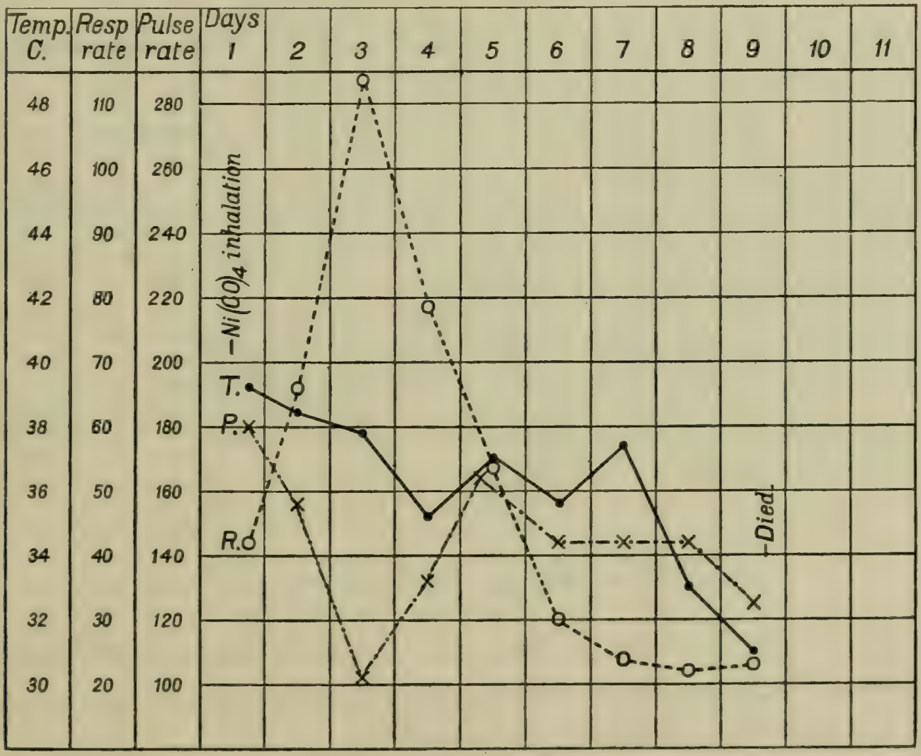

Fig. 4. Chart of Cat 11.

On the 6th day the reflexes became less brisk, and on the 7th day there was distinct paresis with some ataxy of the gait. The signs of consolidation did not increase and on the 7th day a considerable increase in the râles was noted. The breath sounds became faint. The cat died after about 200 hours, during the night of the ninth day. The loss of weight represented over a quarter of the original weight. There was diarrhoea during the last three days.

(vi) *Variations of symptoms in cats.*

Vomiting during the inhalation was frequently seen. In some cases there was little increase in the respiratory rate during the greater part of this period.

The respiratory rate in the subsequent stages varied considerably. The following gives the range of the variations :

Up to 6	hours	after	poisoning	26 to 30	per minute.	
7 to 24	hours	„	„	30 to 48	„	(or more)
25 to 48	„	„	„	60 to 120	„	
49 to 60	„	„	„	70 to 100	„	
61 to 84	„	„	„	70 to 120	„	
85 to 96	„	„	„	70 to 100	„	
97 to 120	„	„	„	60 to 80	„	

The decrease in the rate was marked in some cases, especially when death occurred late. When recovery took place the fall to normal was gradual. During the acute period, laryngeal stridor and a hoarse miaow were often observed.

Death occurred on an average in 88·93 hours in cats which were subjected to the inhalation of 0·04 volume per cent. for 75·5 minutes.

In 1 case, it was delayed to 348 hours.

In 3 cases, it took place between 200 and 250 hours.

In 6 cases, it took place between 100 and 200 hours.

In 4 cases, it took place between 27 and 40 hours and in one case it took place during the inhalation.

(vii) *Poisoning in dogs, guinea-pigs, etc.*

Dogs behave like cats ; they are slightly more susceptible to the poison than the latter. Diarrhoea was present in all the cases. In other respects no differences were noted.

Guinea-pigs proved to be very susceptible to nickel carbonyl vapour. They showed most of the signs and symptoms which are seen in rabbits.

Insects die rapidly in dilute mixtures of the vapour.

A series of experiments was conducted on frog's heart and muscle nerve preparations. The carbonyl was applied in vapour and also in liquid form. No direct effect could be noted. It is however uncertain whether the nickel carbonyl ever reached the muscle cells or nerve fibres, owing to the fact that it is readily dissociated (see Part I) and also to the unfavourable conditions for absorption.

III. PATHOLOGICAL CHANGES.

(i) *Macroscopical.*

As has been stated, rabbits usually assumed a position of orthotonos before death occurred and the cadaver retained this position. In cats, this characteristic was not noted.

Rigor mortis took place normally. No typical external appearances were present after death.

No changes have been seen in the heart after poisoning with nickel carbonyl. In one case, emphysema pericardii was present.

The Lungs.

The changes met with in the lungs consisted of congestion, oedema, haemorrhage and compensatory emphysema.

When death occurred during the first 24 hours, the hyperaemia was often the only change present. In the majority of cases, however, there was fairly well marked oedema and at times a few punctate haemorrhages were seen on the pleural surface and on section. Parenchymatous haemorrhages of considerable size were rare at this stage.

When death occurred during the second day, the hyperaemia was intense. The lungs were freely bathed in a blood-stained fluid. Numerous small and medium sized haemorrhages, not only on the pleural surface, but also pervading the whole organ were common. Large haemorrhages were less frequent. In some cats, patches of consolidation were seen at this stage.

When death took place during the third day, small patches of more or less complete consolidation were usually present. These patches were of dark mulberry colour and exuded blood when squeezed. The haemorrhages which affected considerable areas of lung also gave the appearance of consolidation. They were large or small and were usually scattered over the whole organ, save in those situations in which emphysema existed. The emphysema was best seen at the free edges of the lobes and a characteristic mosaic-like appearance was seen on the pleural surface. Oedema was rarely clearly seen by the naked eye at this stage on account of the other changes which obscured it.

When death took place during the 4th or 5th day, the infiltration with blood-stained fluid, the consolidation and haemorrhagic foci had reached their maximum. A few fresh haemorrhages were found in a few cases but the majority were of older standing. Whole lobes or even whole organs presented an almost uniform mulberry colour and had the consistence of liver. The tissue sank in water. On section, a frothy blood-stained fluid could be expressed.

Pleural effusion was found in one case.

The Bronchial Glands.

In cats, the bronchial glands were seen to undergo changes in keeping with those in the lungs. At first there were some swelling and redness. The colour then became darker and small haemorrhages were seen. Dark specks were seen in the substance after about 36 hours; these specks sometimes had a greenish tinge. Later, the haemorrhages became more intense.

Trachea, Larynx and Bronchi.

In practically all the cases, the mucous membrane of the upper air passages was markedly injected for the first few hours after the inhalation. Haemorrhages were sometimes seen early, but more often they occurred at a later stage. The passages often contained a viscid greenish fluid or else a clear frothy or blood-stained fluid.

Liver, Pancreas, Spleen, Kidneys, Bladder and Genital Organs.

Changes in these organs were seen only on rare occasions. In a few cases, haemorrhages were met with in the kidneys, spleen, pancreas, ovaries, testes and uterus.

Intestines and Stomach.

Haemorrhages were seen at times in the mucous membrane of the stomach and very rarely in that of the intestines. Congestion and ulceration were also met with exceptionally.

Adrenals.

Changes in the adrenals were met with in the majority of the cases. These were first seen after the 2nd day. Small localized or large diffuse haemorrhages were common and general swelling and redness of the organs usually accompanied them. At times there was only congestion. The haemorrhages were usually situated in the medulla of the adrenals.

Brain.

Changes were commonly met with in the brain and were sometimes extensive. The meninges were generally suffused with blood, the vessels were dilated and at times the membranes thickened. In rare cases, some meningeal haemorrhages were seen. The changes were met with from the 2nd day onward and were the more intense the later death took place.

As a rule, the white matter of the brain was the seat of haemorrhages. These were generally minute. They were seen in the *optic thalami, corpus striatum,* internal capsule and *pons varolii,* as well as in other situations. In the medulla and cerebellum, haemorrhages were also seen in a number of cases. Simple congestion was met with frequently. Extensive haemorrhages in the brain of rabbits, cats and dogs, like those observed in the brain of one of the human subjects, who died of plant gas poisoning at the Nickel Works, have not been met with.

The Spinal Cord.

In a few cases, in which the spinal cord was examined, no pathological changes were seen.

The following table shows the frequency of haemorrhages met with in the various organs.

TABLE II.

Showing the frequency of haemorrhages in the various organs.

	Cats (31)	Rabbits (93)
Lungs	100 %	100 %
Liver	6·4	4·3
Spleen	0	2·1
Pancreas	3·2	1·0
Kidneys	0	6·4
Adrenals	83·8	51·6
Stomach	0	9·6
Genital organs	0	5·3
Brain	74·1	37·6

(ii) *Microscopical.*

It is only necessary to describe the microscopical appearances of the lungs, bronchial glands, adrenals and brain, as the changes met with in other organs were rare and corresponded exactly to the macroscopical appearances.

The organs were fixed and hardened in potassium bichromate and acetic acid or Flemming's osmic acid fluid followed by alcohol. Brains were either put through alcohol, bichromate of potassium, formaline or Muller's solution or else fixed by Marchi's method.

The Lungs.

Oedema is the most striking of the early changes. The specimens show a diffuse homogeneous material, which stains more or less deeply with eosin. The fluid is usually situated in the pulmonary tissue, lying like a stroma in the network of the interalveolar tissue. At times, however, it pervades the spaces and may be seen either in the alveoli or even in the bronchioli and bronchi (see Plate VII, Figs. 1 and 2). Often the fluid contains a few blood corpuscles.

Haemorrhages vary considerably in extent. They may be so extensive that the lung structure is quite obliterated. The blood cells are first poured out into the interalveolar tissue and pass into the air vesicles and passages later. In early cases, diapedesis may be marked. The endothelial cells of the capillaries at first appear swollen, then degenerative changes are seen, then the wall gives way at one spot and the blood issues out, gradually enlarging the defect produced in the wall.

Leucocytosis in the vicinity of the capillaries is often extensive in limited areas, and after a time, peribronchitic small cell infiltration is seen. The alveoli and smallest bronchioli become more or less filled with blood cells and the appearance when whole lobules are affected only differs from that of typical broncho-pneumonia by the occasional presence of the homogeneous fluid and haemorrhagic patches. In advanced cases, there is a typical inflammatory process, identical to that of pneumonia, including the polynuclear exudate in the alveoli, pus cells in the bronchi and well marked desquamation of the endothelium. At times, one meets with some proliferation of the bronchial epithelium. In the earlier stages, the polymorphonuclear cells are usually absent and the infiltration is then limited to small round cells.

The Bronchial Glands.

Considerable congestion, small cell infiltration and haemorrhages may be seen quite early. The lymphoid tissue appears at first to be but little affected, since the leucocytosis and haemorrhages are not extensive. Later on more extensive haemorrhages may occur. In some situations minute intracellularly placed granules of dark colour may be seen. At times, the endothelial cells take up red blood corpuscles.

The Adrenals.

The changes met with correspond to those in the lungs. In the earliest stages, the endothelial cells of the capillaries undergo degeneration, then they rupture and the blood cells are poured out into the surrounding tissue. Later on the parenchymatous cells may degenerate secondarily (see Plate VIII, Fig. 5). During the early stages, leucocytosis may be seen in the neighbourhood of the capillaries. Primary changes in the parenchymatous cells cannot be detected, but the fact that haemorrhages frequently obscure the early changes must be taken into account before one can definitely exclude the possibility of their existence.

The Brain.

In this organ, the changes are like those met with in the lungs. The earliest changes seen are found in the walls of the capillaries and small blood vessels. The endothelial cells are swollen, the nuclei are badly stained and the outlines of the cells are not well differentiated. Many of the cells are misshapen. When fixed by osmic acid, fat globules are seen both below the endothelial layer and in the endothelial cells themselves (see Plate VIII, Fig. 6). In a few situations, fat is seen in the lumen of the vessel, in cells lying free. These cells appear to be like endothelial cells and it is probable that they had been cast off.

After the cells have become sufficiently affected, they are no longer capable of withstanding the pressure of the blood and thus the vessel gives way, at first by the failure of one or two cells and later by a number of contiguous cells becoming destroyed or pushed aside. The blood escapes from the vessel in comparatively narrow tracks and the cells are found closely packed together (see Plate VIII, Fig. 7). A considerable amount of leucocytosis is seen in the neighbourhood of the affected capillaries, especially before the rupture takes place. The leucocytes are frequently grouped around nerve ganglion cells and appear to be connected with the changes which take place in the latter, even before a haemorrhage takes place. At first, the Nissl's granules become indistinct and stain weakly. Later on, they become more and more ill defined until they disappear and the contents of the cell appears to be homogeneous (see Plate VIII, Fig. 8). During the later stages, the processes of the ganglion cells can no longer be demonstrated. In the majority of cases, the chromatolysis does not become complete.

Secondary degeneration of the nerve fibres is not often seen in cats and never in rabbits, since the time between the inhalation and death is too short for such processes to be marked. Slight Marchi degeneration was seen in the case of a cat, in which repeated sub-lethal doses of nickel carbonyl were given, with the effect that the cat lived for 22 days, during which time it was continually under the influence of nickel.

IV. ATTEMPT TO TRACE THE NICKEL THROUGH THE ORGANISM.

(i) *Methods of detection.*

The detection of nickel in the various tissues was carried out in two ways. Firstly the organs were ashed and the nickel was determined quantitatively by the dimethyl glyoxime method, described by Harden and myself (1906)[1]. Secondly the nickel was recognized by micro-chemical means. The first method was necessary in order to obtain information with regard to the passage of the nickel and the rate of its passage, while the second method was found only to be available during the earliest stages in the lungs, before the nickel had entered into complex combination.

Considerable difficulty was experienced in working out a satisfactory method of micro-chemical detection of nickel in the tissues. Two reactions were found to be applicable. The first of these is the dimethyl glyoxime reaction, but this has a great disadvantage in that the compound first forms in solution and subsequently crystallizes out. The appearance of the sections, however, was very characteristic, when the preparation was carried out as follows. A rabbit was exposed to a high concentration of nickel carbonyl vapour and on being removed from the poison chamber was at once killed. The lungs were removed and portions were cut fresh by the freezing microtome. The sections were first floated out on a weak solution of sodium triphosphate, to prevent a solution of the nickel, then they were transferred to a solution of dimethyl glyoxime in methyl alcohol, to which a few drops of ammonia had been added and allowed to remain in this solution for several minutes. They were then rapidly passed through water containing a few drops of ammonia and spread out on slides. The specimens were examined either unstained or faintly stained in methylene blue. The nickel appeared as a diffuse red staining of the tissue, which however paled rapidly and from which red needle shaped crystals separated out. The position of these crystals was no indication of the position of the nickel. The other method showed that the temporary appearances observed by means of the first method actually corresponds to the position of the nickel.

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[1] Harden and Armit. *Proc. Roy. Soc.*, 1906.

Another method for the detection of nickel in the tissues was employed. A rabbit was poisoned by inhalation of nickel carbonyl in air for 25 minutes. After an hour during which the nickel carbonyl would be entirely dissociated, it was killed. The lungs were removed and blown up several times with sulphuretted hydrogen for about thirty minutes. They were then blown up with an indifferent gas (carbon dioxide was used), which first passed through a tube immersed in boiling water. The lungs were kept during this stage in a dry vessel at a temperature of 90° C. After the lungs had been heated in this way for three hours, portions were removed and cut by the freezing microtome. The sections were floated out on water, passed through spirit and again floated out on water. They were then spread out on slides and stained with haematoxylin. The nickel was seen as a brownish staining of the tissues. On examining the affected parts with a high power, very minute granules of a brownish colour could be detected. A similar appearance was seen when a solution of the dissociation product of nickel carbonyl in serum (see Part I of this paper) was treated with sulphuretted hydrogen and the serum coagulated by heat was examined under the microscope. The solution of the nickel sulphide in serum is a dark brown almost clear solution, but when seen under a high magnification, it is found to contain minute granules of dark colour.

(ii) *Evidence of what takes place in the lungs.*

Fate of nickel carbonyl when inhaled in the lungs.

In tracing the nickel carbonyl through the organism, it is necessary to enquire what happens at the site of absorption. It is therefore desirable to consider the immediate result both to the carbonyl and to the lungs when the vapour is inhaled. It has already been shown in Part I of this paper, that nickel carbonyl vapour when placed in contact with air and moisture at the temperature of the animal body dissociates rapidly. Evidence that dissociation takes place in the air passages is found in the deposition of a nickel containing substance on the surface of the mucous membranes. In order to follow the process, it is necessary to consider what amount of nickel is available for absorption and also in what way and with what rapidity it is absorbed by the blood.

Direct measurement of the quantity of nickel carbonyl dissociated could not be carried out, on account of the difficulty presented to accurate analysis by the small differences in the volume per centage of the already dilute gas mixture before and after passing through the poison chamber.

Indirect evidence is however obtainable. The maximum amount of nickel carbonyl available is calculated by determining the amount of air mixture which the animal breathes during the experiment. As has been stated, the inhalation of 0·018 volume per cent. of nickel carbonyl vapour in air for 50·5 minutes suffices to kill rabbits. A rabbit weighing 2 kilograms takes in about 600 c.cm. of air per minute under normal conditions. It would therefore breathe about 32 litres during the inhalation. The total amount of nickel carbonyl vapour breathed would therefore be about 5·9 c.cm or 0·045 gram. This would contain about 0·0155 gram of nickel. The total quantity of nickel carbonyl entering the lungs would correspond to about 7½ mgrs. of nickel per kilogram body weight.

The animal would not absorb all the vapour which is contained in the air entering the respiratory passages, and evidence that some of the vapour is not absorbed is found in the fact that the expired air of an animal immediately after the inhalation contains recognizable quantities of nickel carbonyl. The amount mentioned above must therefore be regarded as a store from which the animal will abstract a fraction, which will serve as a lethal dose.

In cats, the inhalation of 0·04 volume per cent. of nickel carbonyl in air for 75·5 minutes proved sufficient to kill. A cat weighing 3 kilograms breathes about 800 c.cm. per minute. This would correspond to about $60\frac{1}{2}$ litres during the inhalation. This is equivalent to about 24·3 c.cm. or 0·184 gram of nickel carbonyl or roughly 64 mgrs. of nickel. The available amount of nickel carbonyl expressed as nickel would therefore not exceed 21 mgrs. per kilogram body weight.

In the case of a cat which was affected with an old pulmonary lesion, probably of tubercular origin, and which died at the end of the inhalation before it could be removed from the poison chamber, 32 mgrs. of nickel were recovered from the lungs after ashing, while the blood was found to be free from nickel. The cat weighed 3800 grams, so that 8·4 mgrs. of nickel per kilogram body weight was found. In other cases, the amount recovered from the lungs was less than this amount, since absorption from the lungs takes place early. The average amount recovered was about 10 mgrs. The amount found in the blood of cats immediately after inhalation varied up to 3·75 mgrs. per 100 grams of blood. Thus in cats weighing up to 3 kilograms, from 20 to 22 mgrs. of nickel has been accounted for in the lungs and blood, while small quantities would probably be present in other tissues (e.g. the bronchial glands usually contain small quantities early). Making some allowance for this and having regard to the result of the experiment in which 32 mgrs. was recovered, it may be assumed that after inhaling 0·04 volume per cent. of nickel carbonyl vapour in air for $75\frac{1}{2}$ minutes, cats are able to dissociate an amount of nickel carbonyl representing not less than $8\frac{1}{2}$ mgrs. of nickel per kilogram body weight. This would correspond to between $\frac{1}{3}$ and $\frac{1}{2}$ of the total available amount. In rabbits, up to $3\frac{1}{2}$ mgrs. of nickel per kilogram body weight has been accounted for in the same way immediately after inhalation. The absorption of about one half of the inspired quantity of a toxic gas has been met with in the case of carbon monoxide (Haldane, 1895).

It has already been stated that from the earliest stage of nickel carbonyl poisoning, the lungs undergo changes. It is uncertain whether the vapour as such acts as an irritant. Nickel carbonyl is dissociated into carbon monoxide and a nickel containing product which is probably a hydrated sub-carbonate of nickel (see Part I, p. 541 et seq.). The quantity of the carbon monoxide is too small to produce any appreciable effect, since the amount of carbon monoxide liberated from the nickel carbonyl corresponding to $8\frac{1}{2}$ mgrs. of nickel would be less than $9\frac{1}{2}$ c.cm., and this small amount would be available for absorption during the 75½ minutes (see also Part I, p. 528). The nickel containing substance is gradually dissolved by the tissue fluids and the dissolved nickel acts locally upon the peribronchial and alveolar tissue as well as on the endothelial cells of the capillaries producing the changes which have already been described. The production of a serous effusion or oedema hastens the solution of the deposited nickel compound. At first, the nickel containing product of dissociation is simply dissolved, without any alteration of its chemical constitution, but after the course of some hours, it becomes acted on by the proteid material and a more complex nickel combination results. The evidence of this is found in the fact that at first the red compound forms with dimethyl glyoxime readily, but later on this combination only takes place slowly, and after about 24 to 36 hours although there is still nickel in the lungs, as demonstrated by analysis after ashing, neither the dimethyl glyoxime compound nor the sulphide can be formed.

At times, the absorption takes place very rapidly and in one case of a small rabbit, less than 1 mgr. of nickel was found in the lungs immediately after poisoning. The process of absorption however takes some time to complete and 3 mgrs. has been found 24 hours and 1 mgr. 48 hours after the inhalation in rabbits. From this time onwards only small traces could be detected and although 0·035 mgr. was found in one rabbit after 96 hours, the nickel was usually completely removed after about 2½ days.

The microscopical appearances assist in determining the way in which the nickel is taken up. The free surfaces and the tissue immediately surrounding the surface of the bronchi, bronchioli and alveoli show red or brownish staining when the sections have been treated by the dimethyl glyoxime or sulphide methods. The study of the dissociation of nickel carbonyl described in Part I has shown that under the conditions of temperature, moisture and carbon dioxide obtaining in the lungs, the dissociation product is formed, which is slightly soluble in water and in solutions of phosphates. It is thus clear that the nickel subcarbonate is deposited on the respiratory mucous surfaces and is gradually dissolved from this situation by the tissue fluids. This accounts for the intense staining at the free edges and the diffuse staining in the neighbouring tissues.

(iii) *The absorption of the nickel by the blood.*

Evidence of the absorption of the nickel deposited on the respiratory surface and dissolved from this situation by the tissue fluids may be obtained from (1) the presence of nickel in the blood during the early stages, (2) the gradual disappearance of the nickel from the lungs, (3) the appearance of nickel in other organs, and (4) the appearances of the micro-chemical tests in the lungs during the early stages.

(a) The fate of nickel carbonyl in artificial profusion experiments.

In following out the fate of nickel carbonyl after inhalation, a short series of experiments with artificial perfusion of the lungs was carried out. It was found that when cats' lungs were employed, the experiments were unsatisfactory, as the tissues became oedematous early and thus terminated the experiment before sufficient time had elapsed. Dogs' lungs were therefore used.

The experiments were carried out with an apparatus devised by Martin and Embley (1905)[1]. The lungs were removed and the respiration was maintained by inflating them rhythmically with air containing varying amounts of nickel carbonyl vapour. The animal's own blood defibrinated and kept at 37° C. was used, which was circulated through the lungs. Samples of blood were taken from time to time, and ashed to determine the quantity of nickel which had been taken up. At the end of the experiments, the lungs were also ashed and examined quantitatively for nickel.

The results of two experiments conducted in this way were as follows:

TABLE III.

	Duration in mins.	Amount of air used (litres)	Amount of $Ni(CO)_4$ used (grams)	Vol. % of $Ni(CO)_4$ vapour	Blood (grams)	Mgrs. Ni per 100 grams blood	Mgrs. Ni in lungs
1.	90	891	0·9436	0·014	300	(a) 0·05	
						(b) 0·019	
						(c) 0·037	3·00
2.	50	450	8·8188	0·251	250	(a) 1·75	
						(b) 1·91	25·87

From these figures it would appear that although the greater part of the nickel is left in the lungs, some of it is taken up by the blood. The amount in the blood is larger the more concentrated the nickel carbonyl vapour. In the first experiment about 0·1 mgr. of nickel appeared in the total quantity of blood used and in the second experiment about 4·5 mgrs. These quantities are extremely small as compared with the 3 mgrs. and 25·8 mgrs. found respectively in the lungs.

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[1] Martin and Embley. "The action of anaesthetic quantities of chloroform upon the blood vessels of the bowel and kidney; with an account of an artificial circulation apparatus." *Journ. Phys.* xxxii. p. 147. 1905.

The first sample taken in the second experiment was found to contain carbon monoxide corresponding to 21·4 % of the quantity necessary to saturate. This is quite consistent with what has already been said on the subject of carbon monoxide absorption (see Part I, p. 529), since the concentration of the nickel carbonyl vapour was considerably greater than was employed in the inhalation experiments on animals.

The conditions of these experiments are not exactly similar to those of inhalation by a living animal. The surface of the lung was considerably cooler and the tissues were in a dying condition. The experiments, however, show that dissociation of nickel carbonyl takes place rapidly in the lungs at body temperature and that the nickel is at first deposited in the lungs to be gradually dissolved and carried away by the blood. In the experiments it is possible that some nickel carbonyl went into solution in the blood but it would be rapidly dissociated before it had time to leave the blood again. The fact that the lungs became oedematous and thus terminated the experiments when high concentrations were used sooner than when lower concentrations were used points to a direct toxic action of the nickel on the tissue.

(b) Rate of absorption of nickel from the lung.

The blood, as has already been shown, contains varying quantities of nickel during the early stages. At times no nickel was recovered after ashing small samples within the first day, but as a rule, especially when 50 grams were ashed, easily detectable amounts were regained. The rate of the removal of nickel from the pulmonary tissue will depend on the conditions of the circulation obtaining for the time. The presence of a finely divided nickel compound on the mucous surface produces an inflammatory reaction. The leucocytes and inflammatory cells increase the density of the organs. A serous exudation is thrown out and the air passages become more and more encroached upon, and as a result parts of the respiratory surface become obliterated. It has further been shown that nickel coming into contact with the capillary walls causes changes of a fatty degeneration (this was best demonstrated in the vessels of the brain). The cells give way and one of the results of the haemorrhages which ensue is that the organs become still denser. The circulation through the lungs is thus slowed down by the resistance of the solid organ and by the mechanical interference to the circulation brought about by the rupture of the capillaries and consequent coagulation. Owing to the slowing of the blood stream, the nickel finds it increasingly difficult to get out, and as a result nickel can still be found in the lungs at the end of 60 hours or at times even later.

The quantity of nickel during the second and third day which is present in the blood at any given time must be extremely small, and it is therefore not surprising that none was detected during the second day in any of the analyses. As will be mentioned later, there is reason to believe that nickel is rapidly claimed by other tissues, and this would therefore lessen the quantity present in the blood at any given time. Little by little however the nickel is washed out of the lungs into the blood stream. A certain amount finds its way into the bronchial glands through the lymphatic channels. It can therefore be said that the amount taken up by the blood and available for other tissues at any given time is inversely proportionate to the acuteness of the onset of the pneumonic processes. The more slowly the consolidation of the lungs takes place, the larger will be the amount of nickel which enters the circulation at any one time.

(iv) *Deposition of nickel in the tissues and organs.*

It has been stated that evidence of the absorption of the nickel was obtained by the appearance of nickel in other organs. The various organs and tissues were analysed for nickel at the various stages of the poisoning, but no nickel was detected at any time in the liver, spleen, pancreas, muscles or bile. A very small trace was found on one occasion in the heart immediately after the inhalation, but this was probably due to the accidental inclusion of some blood clot. Nickel was found in the bronchial glands within a short time after poisoning. In the kidneys, nickel was only found at a late stage. This fact will be referred to when the subject of the elimination of the nickel is discussed. The adrenals contained nickel from the 36th hour after inhalation onward. As a rule, the quantity was very small and at the later stages, it did not exceed 0·02 or 0·03 mgr. It was also found in the brain regularly after the end of the first day until the end of the fourth day. A table is appended which shows the position of the nickel during the successive stages of the poisoning.

Evidence of selective absorption by certain tissues.

The results of these analyses point to a selection on the part of the adrenals and brain. The nickel must be available for all the organs, but save for an occasional lesion in other organs, there is no evidence of the presence of the toxic metal in them. The blood yields up its nickel to the adrenals, and brain during the course of the second, third and fourth days. A certain amount of nickel is found in the bronchial glands early, but this is more probably due to the absorption of the nickel either in solution or even in solid form in the bodies of leucocytes through the lymphatic channels. The presence of nickel in the kidneys and the occurrence of pathological lesions in these organs may be neglected for the time being. It must also be pointed out that the lesions found in the organs are practically limited to the lungs, adrenals and brain, both in nickel carbonyl poisoning and also, as will be shown later, in poisoning by nickel and its other compounds. The urine contained nickel from the 24th hour till the fourth day or later, and this would further support the contention that the nickel does not remain long in the organs but passes through them, producing damage during a short stay. One is therefore unable to present evidence typical of selective absorption, namely a definite proportion between the quantity of the nickel and the mass of the organ. The maximum amount of nickel found in the brain was about 0·01 % of the weight of the organ and 0·006 % of the weight of the organ in the adrenals. In spite of this, however, the failure of detecting any nickel in the liver and other organs and the localisation of the pathological lesions must be regarded as evidence of selection on the part of the adrenals and brain.

TABLE IV.

Showing the localisation of the nickel in the tissues and organs at varying times.

	0—12 hrs.	12—24 hrs.	24—36 hrs.	36—48 hrs.	48—60 hrs.	60—70 hrs.	Later
Lungs	+ + +	+ + +	+ +	+ +	+	0	0
Bronchial glands	+	+ +	+ +	+ +	...	0	...
Stomach	...	...	...	...	0	0	0
Adrenals	...	0	0	+ +	+ +	+ +	+
Brain	0	×	×	+ +	+ + +	+ +	+
Kidneys	0	...	0	...	+	+ +	+
Blood	+ +	+	0	0	+ +	+ +	+
Milk	...	...	...	...	+ +	...	...
Faeces	...	...	0	...	+ +	+ +	+ +
Urine	0	0	+	+ +	+ +	+ + +	+ +
Bronchial secretion	+ +	+ +	+ +	...	...	...	...

+ indicates present at times. + + indicates present.
+ + + indicates present in comparatively large quantities.
0 indicates absent. × indicates present on rare occasions.

(v) *Elimination.*

Nickel is excreted in the urine and faeces. On one occasion the milk of a lactating cat was examined and was found to contain nickel on the third day. No nickel was found in the stomach contents, save once in the case of a rabbit in which death occurred early. It is however probable that some bronchial secretion had been swallowed, as this contained nickel.

About 75% of the nickel excreted is found in the urine in rabbits, while in cats over one half of the total quantity is got rid of in this way. The rate of elimination through the kidneys was found to be very irregular, the maximum however being usually reached on the fourth to the fifth day. When the urine contained comparatively large quantities of nickel, some metal was also found in the kidneys. The changes found in these organs were probably produced at this stage, since no congestion or haemorrhage was met with when death took place before the end of 60 hours after the inhalation.

The quantities of nickel in the faeces also reached a maximum about the fourth or fifth day. Diarrhoea usually set in about this period and it was found that the amount of nickel was always greater when the stools were fluid. The faeces then assumed a darkish colour. The nickel cannot be extracted by the action of water or dilute acids and it is probably present in the form of sulphide.

At times death took place after all the nickel had been excreted.

The nickel in the urine appeared to be in a condition of complex combination. Urine containing nickel does not give the dimethyl glyoxime or the sulphide reactions, but after boiling with concentrated nitric acid and neutralising with ammonia both reactions can be obtained. The nickel cannot be precipitated by heat. The urine was subjected to dialysis. After 24 hours, the process was incomplete. In one case, 90 c.cm. of urine was dialysed with 400 c.cm. of water. At the end of 24 hours, 30% had passed through the membrane, while nearly 60% was left inside the skin, the remainder having been absorbed into the membrane. This subject will be again referred to when the excretion of metal in cobalt poisoning is discussed.

An attempt was made to remove a possible organic compound of nickel from the tissues by means of certain solvents (aether, alcohol, saline fluid and water). While aether failed to extract any nickel, the other solvents removed part of the metal. The amounts extracted were very irregular and in no case was the whole amount extracted.

V. POISONING WITH NICKEL AND NICKEL SALTS.

Since nickel carbonyl poisoning is actually poisoning by nickel, in which the metal is applied to the enormous area of the respiratory surface, the symptoms and pathological changes met with in nickel poisonings should correspond with those met with in nickel carbonyl poisoning, allowance being made for the mode of entry.

Laborde and Riche (1888) found that when the soluble salts of nickel are given by mouth, vomiting is produced, but even when 3 grams of nickel sulphate is used, death does not follow. A. Riche (1888) however met with late death after feeding with food impregnated with nickel sulphate in dogs. Death has been seen when the sulphate was injected subcutaneously and intravenously by these two observers. The lethal dose of nickel sulphate applied in this way was found to be 0·0655 gram for rabbits, while 0·378 gram was required for dogs.

Nickel sulphate however is an extremely unsuitable salt for toxicological experiments, as it coagulates albumin and whether injected subcutaneously or intravenously, it is only absorbed very slowly and incompletely owing to the small surface for absorption of the coagulated mass. According to Stuart (1884) the lethal dose of nickel (given as NiO) for rabbits and cats is about 8 mgrs. per kilogram body weight, while for dogs it is higher.

The nickel compounds dealt with in the present investigation are the carbonate, the tri-phosphate, the acid phosphate, the pyro-phosphate, the hydrate, the sulphate, the chloride, the acetate, the ferro-cyanide, the sulphide, the tartrate and the potassium albuminate. Metallic nickel in a state of very fine division was also used. The animals experimented on were guinea-pigs, rabbits and cats. The various preparations were introduced by subcutaneous, intravenous and intraperitoneal injection.

1. Subcutaneous injection.

0·0023 gram of nickel in the form of sulphate, phosphate, ferrocyanide and chloride killed guinea-pigs. The same dose of nickel in the form of acetate also killed. This corresponds to about 6½ mgrs. of nickel per kilogram body weight. 0·008 gram of nickel as carbonate was required to kill guinea-pigs. The lethal dose by this method for rabbits with the soluble salts was found to be between 7 and 8 mgrs. per kilogram body weight. Cats died after the injection of 0·23 to 0·03 gram of nickel as nickel sulphate. The lethal dose for cats per kilogram body weight thus appears to be from 9 to 16 mgrs. calculated as nickel.

2. Intravenous injections.

A few experiments were conducted with intravenous injection but the method was discarded when it was found that the soluble salts, such as the sulphate and chloride, and the insoluble salts, such as the hydrate and carbonate, mostly blocked up a small vessel and unless injected in comparatively large quantities or in minimal quantities in several situations, rarely produced symptoms.

3. Intraperitoneal injection.

The insoluble salts were freshly precipitated and injected with aseptic precautions into the peritoneal cavity. Especial attention was paid to the behaviour of the physically indifferent salts, such as the hydrate, carbonate and phosphates. Guinea-pigs died after receiving 5 mgrs. per kilogram body weight, but after death some unabsorbed salt was found in the peritoneum. When larger doses of these salts were used (e.g. 12 mgrs. of nickel) the unabsorbed material could be recovered to a great extent and estimated after dissolving in acetic acid. From these estimations, it would appear that death follows after the absorption of between 3 and 4 mgrs. of nickel per kilogram body weight.

The tartrate was further used to determine the lethal dose, and it was found that rabbits died after the injection of 8 mgrs. of nickel per kilogram body weight. It was noted however that the absorption of the tartrate is more complicated than that of the salts containing free nickel ions, and from the experiments conducted with the acetate, hydrate and phosphate, it appears that the minimum lethal dose for rabbits is less than 7 mgrs. of nickel.

Nickel sulphide was injected into the peritoneal cavity. 0·025 mgr. (calculated as nickel) led to death in two out of three guinea-pigs. After death a large quantity of black material was found in part free and in part adherent to the surface of the peritoneum. Around the adherent particles, a distinct greenish colour was seen at the periphery. The tissue was removed and treated with acetic acid. Nickel was found in the filtrate, showing that the sulphide had been reduced and that the nickel had combined with oxygen or carbon dioxide.

Metallic nickel was prepared from the hydrate or from the oxalate by drying and reducing in hydrogen at about 260° to 280° C. and sealing up the tubes in which the reduction had been carried out. The metal prepared in this way existed in a state of very fine division and was pyrophoric (i.e. took fire spontaneously when exposed to oxygen). Some of the samples worked with were less finely divided than others. The end of the tube was broken off and a little well boiled physiological saline solution was immediately run into the tube, before the hydrogen in the tube had time to diffuse. The metal was then suspended in the fluid by rapid rotatory movements and sucked up into a glass syringe, without any contact with air. The suspension was injected into the peritoneal cavity of guinea-pigs and rabbits.

The exact dosage was difficult to determine owing to two circumstances. Firstly a little material was always left in the tube, syringe and needle, and secondly after death a varying amount chiefly comprising the coarser particles and clumps which had formed was found unabsorbed. In guinea-pigs, the quantity injected varied between 12 and 28 mgrs. and all the animals thus treated died save a few in which metal which was not very finely divided was injected. 28 mgrs. of nickel in a state of only moderately fine division failed to kill a rabbit, while 30 mgrs. of the same sample killed a second rabbit. Post mortem, relatively large quantities of black material clumped in patches were found in the peritoneum. In every case it was evident that only a part of the nickel injected had been absorbed.

The absorption of the nickel was studied further in these cases. The mesentery which contained small macroscopically visible deposits of black material was stretched on cork discs, fixed, transferred to cover glasses, stained and mounted. In these one could see that the very minutest particles had been taken up by the polynuclear cells and to a less extent by the endothelial cells and by some of the red blood corpuscles. Phagocytosis of the red cell was also seen in situations where the red cells had taken up nickel particles. Nickel granules were further seen in the walls of the lymph spaces, lying free. No granules either intracellularly or extracellularly placed were seen in the blood vessels. The taking up of the nickel appeared to be most extensive about 24 hours after the injection but even as late as 70 hours it could still be seen.

Experiments were further carried out *in vitro*. It was found that pyrophoric nickel, nickel sesquioxide and nickel sulphide in fine state of division are readily taken up by the polymorphonuclear cells.

Symptoms and pathological changes in nickel poisoning.

Guinea-pigs poisoned by nickel and nickel salts all exhibited the same symptoms and post mortem changes. The former consisted in dyspnoea, paresis, at times paralysis and later diarrhoea, cyanosis and convulsions. The lungs showed haemorrhages, compensatory emphysema and some oedema and the adrenals showed haemorrhages.

Rabbits exhibited symptoms like those of nickel carbonyl poisoning, with the exception of the early dyspnoea and giddiness. The respiratory rate became increased after a few hours, the temperature began to fall, the appetite was lost and the animal became dull and listless. These symptoms increased steadily until the dyspnoea became intense and was accompanied by cyanosis. Paresis was only seen in a few cases. Diarrhoea was usually seen after about 24 to 36 hours and death followed in the course of from a few hours to three or four days. The temperature sank to a low level before death.

In cats, the subcutaneous application of soluble salts produced characteristic symptoms. At first general illness without any definite signs was seen, later the appetite was lost, the weight fell and vomiting and diarrhoea set in. Paresis or even paralysis was noted and the gait became ataxic. Tremors of the anterior extremities and of the head occurred. The respiratory rate increased within a short time. Death occurred within the first 12 hours when large doses were injected, while when smaller doses were employed, it was delayed for some days but not exceeding eight days.

Post mortem the lungs, adrenals and brain showed similar changes to those seen after nickel carbonyl poisoning. The pulmonary changes were not so extensive or intense as those met with in nickel carbonyl poisoning, presumably because the whole quantity of the metal does not pass through the lungs as it does when the vapour is inhaled. When death took place late, large areas of lung tissue were affected, and it was impossible to distinguish the sections from those of nickel carbonyl poisoning. The kidneys were more frequently affected in poisoning with the soluble salts of nickel especially when these were given intravenously.

VI. COMPARISON WITH IRON AND COBALT POISONING.

(i) *Iron carbonyl poisoning.*

As it is proposed to record the results of experiments conducted with iron carbonyl, pyrophoric iron and iron salts as well as with cobalt and its salts elsewhere, only brief reference to these experiments will be made in this place. It was found that 0·025 volume per cent. of iron carbonyl in air inhaled for 45·5 minutes (after deducting 14·5 minutes, see p. 567) killed rabbits. This yields a maximum quantity of iron of about 17 mgrs. It thus appears that iron carbonyl is less toxic than nickel carbonyl. On the other hand, death takes place slightly more rapidly, the average length of life after poisoning with minimal quantities being 40·4 hours as against 69·3 hours after poisoning with nickel carbonyl.

The symptoms of iron carbonyl poisoning showed considerable similarity with those of nickel carbonyl. Beside the respiratory distress, dyspnoea, cyanosis, loss of weight and fall in temperature, coarse tremors were noted and also spastic gait and paresis of the hind extremities. The heart beat was frequently irregular and intermittency of the beat was noted. The physical signs of consolidation of the lungs were detected earlier than in nickel carbonyl poisoning. Diarrhoea was not present in any of the cases, and when recovery followed the poisoning, a peculiarity of the gait persisted for some time.

The pathological changes met with were of the same type as those of nickel carbonyl poisoning, but all the organs were affected without any preference. In the lungs, the pneumonic process was often intense and not infrequently enormous haemorrhagic infiltration was seen (see Plate VII, Fig. 3). No essential difference between the effects of the two carbonyls upon the lungs could be ascertained. The spleen, kidneys, stomach, intestines and other organs mostly showed haemorrhages and degeneration of parenchymatous cells, while the adrenals were only occasionally affected. The heart was generally dilated and haemorrhages were found in the walls. The brain also showed haemorrhages similar to those of nickel carbonyl poisoning.

The faeces were rarely dark in colour, save when the symptoms lasted for a long time, and diarrhoea was never met with. Iron was found in the faeces and urine. In the urine, it existed partly as a ferric and partly as a ferrous compound.

Comparative experiments with iron and iron salts were carried out in the same way as was done with nickel and nickel salts. The symptoms and pathological changes of iron and iron salt poisoning corresponded to those of iron carbonyl poisoning. The lethal dose of iron when given by intraperitoneal injection proved to be about 20 mgrs. per kilogram body weight. It could be shown that the ferric condition of the metal was retained in the excretion when a ferric compound was used, and the ferrous condition when a ferrous salt was introduced. Micro-chemical examination however showed that in the tissues, free iron ions were present, even if the compound introduced was in a condition of complex combination.

Cobalt was also studied in a similar manner. From these experi ments, it could be shown that no essential difference between poisoning with nickel and poisoning with cobalt exists. On Plate VII, Fig. 4 the changes met with in the lungs after subcutaneous injection with cobalt chloride are shown, and from this it will be seen that all the characteristics of nickel poisoning are present. The only means of determining between the two conditions is by chemical analysis and by the appearance of the urine. The lethal dose of cobalt is higher than that of nickel, rabbits dying after the absorption of from 15 to 18 mgrs. per kilogram body weight, when applied intraperitoneally.

The urine of cobalt poisoning presents a peculiar colour. This was first described by Stuart. The first traces of the colour was seen within 15 minutes of the intraperitoneal injection of the tartrate of cobalt. As a rule, the colour attained its maximum during the course of the second day, but traces were seen during the following two or three days. The colour is a characteristic brown one. As this colour proved to be an easy index of the presence of cobalt in the urine, urine containing it was carefully studied. It was found that the quantity of cobalt varied in proportion with the intensity of the colour. When subjected to dialysis, the cobalt like the nickel passed very slowly through the membrane and for a considerable time during the dialysis a proportion was adsorbed into the membrane. The colour of the urine paled as the cobalt passed into and through the membrane. Boiling did not precipitate the metal. Lead acetate precipitated part of the cobalt but the total quantity could not be thrown out of solution by this means. Silver nitrate also precipitated part of the metal. In both cases, the colour became paler in correspondence with the amount of precipitation of metal. The cobalt containing urine did not form a sulphide on the addition of ammonium sulphide, but after boiling with nitric acid and neutralising with ammonia, the sulphide formed quite readily. 50 c.cm. of urine containing 17 mgrs. of cobalt was placed in two small cells connected with a syphon and a weak electric current was allowed to pass through the solution by means of platinum electrodes. After 24 hours, the syphon was clamped off in the middle, and the fluid in each half was emptied into the corresponding cell. The electrodes were washed with hydrochloric acid. It was found that the cobalt had not been ascertainably deposited on either electrode and that the quantity of metal was equally distributed between the two cells. A solution of cobalt chloride of the same strength was submitted to the same procedure. The cell corresponding to the negative pole contained over 10 mgrs. of cobalt while that corresponding to the positive electrode contained just under 7 mgrs.

The negative electrode was stained brown and 1·5 mgrs. of cobalt was dissolved off it, while the positive electrode was free from cobalt deposit. It thus appears that the cobalt in the urine is in a condition of complex combination and is not present as free cobalt ions. The behaviour of the urine is greatly in favour of the brown colour being due to a cobalt compound.

Pyrophoric reduced cobalt was suspended in serum and kept sealed up for many weeks, but no brown compound formed. When a cobalt salt such as the chloride is added to normal urine, no brown compound results and the cobalt can be combined with sulphur to form the sulphide, by adding ammonium sulphide without any previous heating with nitric acid. Several attempts were made to precipitate the compound or to crystallise it out of solution by the addition of various reagents, but without success.

VII. THERAPEUTIC EXPERIMENTS IN NICKEL CARBONYL POISONING.

It has been suggested that the administration of gases and other substances, which would dissociate nickel carbonyl and deposit nickel in an insoluble form, might lead to recovery after poisoning. The foregoing account however of the events occurring in nickel carbonyl poisoning makes it clear that attempts to convert the nickel compound into an insoluble one within the animal body will not alter the course of poisoning, as this has already taken place. It has been shown that even nickel sulphide is dissociated in the tissues and acts toxically. However it was considered necessary to conduct experiments in this direction, but the results coincide with the theoretical conclusions arrived at. Especial attention was paid to experiments aiming at the formation of the sesquioxide of nickel with bromine, as one of the assistants in Dr Mond's laboratories asserted that he always found relief in the inhalation of bromine after he had been exposed to nickel carbonyl vapour. No animals were saved by this treatment. The same may be said of chlorine vapour. The compounds used in these experiments included the acid phosphate of sodium, the triphosphate of sodium and the pyrophosphate of sodium, sulphuretted hydrogen and ammonium sulphide (poly-sulphides), the ferri and ferro-cyanide of potassium, the cacodylate of sodium and atoxyl, etc. In none of the experiments was a curative action discernible, and it must be assumed that either the more insoluble compound was not formed or that if it was formed it was again dissociated.

Better results attended a purely symptomatic treatment. The object aimed at was to keep the animals under the influence of narcotics and sedatives in order to slow down the circulation and respiration and thus to slow down the rate of absorption of the nickel. It was noticed that in some of the untreated animals, recovery had taken place when the animals remained quite quiet, while others which were apparently less severely affected died if they indulged in exercise during the early stages of poisoning. Morphine was found to be unsuitable. Veronal (diethyl malonyl urea) too did not lead to satisfactory results. A series of experiments with neuronal (bromine diethyl acetamine) showed that if given in doses just sufficient to keep the animals drowsy the fatal result could be averted in a considerable number, provided that only minimal lethal doses had been applied. When the dose of nickel was increased, the treatment failed to save any animals. Twenty-nine cats were exposed to 0·04 volume per cent. of nickel carbonyl vapour in air for 7·55 minutes. Sixteen were treated with neuronal and 13 served as controls. Of the treated cats 9 died and 7 recovered giving 43·7 per cent. of recoveries while of the controls 11 died and 2 recovered, giving 15·3 per cent. of recoveries. The drug proved to be quite harmless, which could not be said of veronal and morphine, and it was found that it was quite easy to regulate the dosage, so that the cats were only drowsy.

One is therefore justified, in the absence of a better form of treatment, to suggest that any cases of poisoning with nickel carbonyl should be treated by complete rest in bed, and small doses of neuronal (starting with 0·5 gram) repeated until somnolence begins to manifest itself.

Treatment only proved of value in cases when an amount of nickel carbonyl which was about a fatal dose had been inhaled, so that it is imperative to adopt preventive measures in places where nickel carbonyl is dealt with. That this is possible has been demonstrated by the fact that since 1904, no cases of poisoning have occurred at the Mond Nickel Works in Clydach, where nickel carbonyl is being produced in large quantities. At these works, the precautions taken to prevent escapes, and also the maintenance of a violent upward draught, effectually prevent an accumulation of poisonous vapour.

VIII. SUMMARY AND CONCLUSIONS.

Nickel carbonyl poisoning is a particular instance of nickel poisoning.

The lethal dose of nickel varies according to the method of application. When applied by subcutaneous injection, the physical condition of the compound influences the rate of absorption and therefore relatively large quantities may be required. In rabbits, the lethal dose is about 7½ mgrs. per kilogram body weight under the most favourable conditions when applied subcutaneously. In cats it is about 12½ mgrs. per kilogram body weight. When applied intraperitoneally, the absorbing surface is considerably larger and consequently the dose required to kill is smaller. In rabbits it is less than 7 mgrs. When applied in the form of nickel carbonyl vapour one meets with the most favourable conditions for rapid absorption and the dose is therefore still smaller. Rabbits die after the absorption of between 3 and 4 mgrs., while cats die after absorbing about 8½ mgrs. per kilogram body weight.

In the lungs, nickel carbonyl is dissociated and a nickel compound, probably the hydrated sub-carbonate, is deposited on the respiratory surface.

The nickel is dissolved from the respiratory surface by the tissue fluids and is then taken up by the blood.

Some of the nickel finds its way directly through the lymphatic channels into the bronchial glands.

In the dissolved condition, the nickel enters into complex combination with some constituent of the body.

The nickel is carried by the blood to the tissues, but a selective absorption is exercised by the brain and adrenals. In the case of other forms of nickel poisoning, the lungs also exert this specific selection. The nickel only stays for a short time in these organs.

The specific pathological changes which are produced by nickel in these organs are primarily a degeneration of the endothelial cells of the capillary vessels. It is possible that some further primary action is exercised on the ganglion cells in the brain and on the parenchyma cells of the adrenals.

The haemorrhages follow as the result of the fatty degeneration of the vessel walls and secondary changes result from the effects of the haemorrhages.

The nickel is excreted by the kidneys and intestines.

The method of poisoning with iron carbonyl is similar to that of nickel poisoning, but the amount necessary to kill in the former case is larger.

Iron carbonyl poisoning like nickel carbonyl poisoning is merely a specific instance of metallic poisoning.

Iron acts in a similar manner to nickel on the walls of capillary vessels, but no evidence of selection by any special tissues was obtained.

Cobalt has a toxicological action which is identical to that of nickel. The lethal dose however is higher than that of nickel and lower than that of iron.

After the inhalation of a quantity of nickel or iron carbonyl which is greater than the minimum required to kill, no form of treatment was found to avert death.

It is with much pleasure that I again express my gratitude to Dr Ludwig Mond, F.R.S., for having rendered this investigation possible, by defraying all the expenses, and by giving me the benefit of his advice.

I further desire cordially to thank Dr C. J. Martin, F.R.S., and the other members of the Staff of the Lister Institute, who have at all times been ready and willing to assist me. The work has necessitated incursions into several branches of science, and has required the acquisition of a variety of methods. I have made free use of their kind collegiality and am glad to avail myself of this opportunity of recording my indebtedness.

The literature of the subject has been given in Part I, q.v.

DESCRIPTION OF PLATES.

PLATE VII.

Fig. 1. Section of lung of rabbit, poisoned by $Ni(CO)_4$, showing intra-alveolar haemorrhage, oedema and fibrinous exudation; intra-bronchial exudation with proliferation of epithelial cells; cellular infiltration of the peribronchial tissue can also be seen. (Leitz Oc. 5, Obj. 3.)

Fig. 2. Same specimen under higher magnification. The oedema and haemorrhage are more clearly seen. Degeneration of the alveolar cells is present. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

Fig. 3. Section of lung of rabbit poisoned with $Fe(CO)_5$, showing enormous haemorrhagic infiltration. The alveolar structure has been partially broken up and the cells are degenerate. Some leucocytosis is present. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

Fig. 4. Section of lung of guinea-pig poisoned by subcutaneous injection of $CoCl_2$. Showing well-marked oedema and fibrinous exudation, inter- and intra-alveolar haemorrhage, with breaking up of the alveolar structure. (Leitz Oc. 1, Obj. $\frac{1}{12}$ oil immers.)

PLATE VIII.

Fig. 5. Section of supra-renal gland of rabbit poisoned with $Ni(CO)_4$ vapour. Well-marked haemorrhagic infiltration and early degeneration of the nuclei of the parenchymatous cells. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

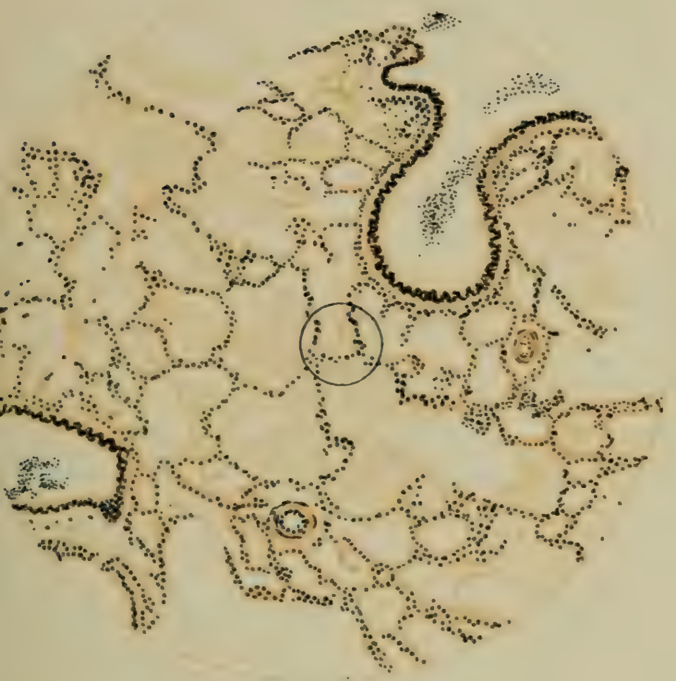
Fig. 6. Section of brain (close to the optic thalamus) of cat, poisoned by $Ni(CO_4)$ vapour. Fixed by Marchi's osmic acid fluid and stained with haematoxylin and eosin. The specimen shows a small vein in transverse section. The wall of the vessel shows well-marked fatty degeneration. The fat globules are situated partly in the sub-endothelial space and partly in the endothelial cells. The latter are swollen, misshapen and poor in chromatin. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

Fig. 7. Section of medulla oblongata of rabbit poisoned with $Ni(CO)_4$ vapour, showing a capillary blood vessel giving way. The degenerative changes in the endothelial cells of the capillary wall are advanced; those cells which are still recognizable are swollen, indistinct and contain little chromatin. There is some leucocytosis in the neighbourhood of the capillary. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

Fig. 8. Section of brain (mid-cerebrum) of rabbit poisoned with $Ni(CO)_4$ vapour, showing chromatolysis of Nissl's granules in the ganglion cells. Many of the processes are broken off and the cells have become misshapen and have lost chromatin. Leucocytosis is also present. The vessel seen has not yet ruptured. (Leitz Oc. 3, Obj. $\frac{1}{12}$ oil immers.)

PLATE IX.

For description see text, p. 566.



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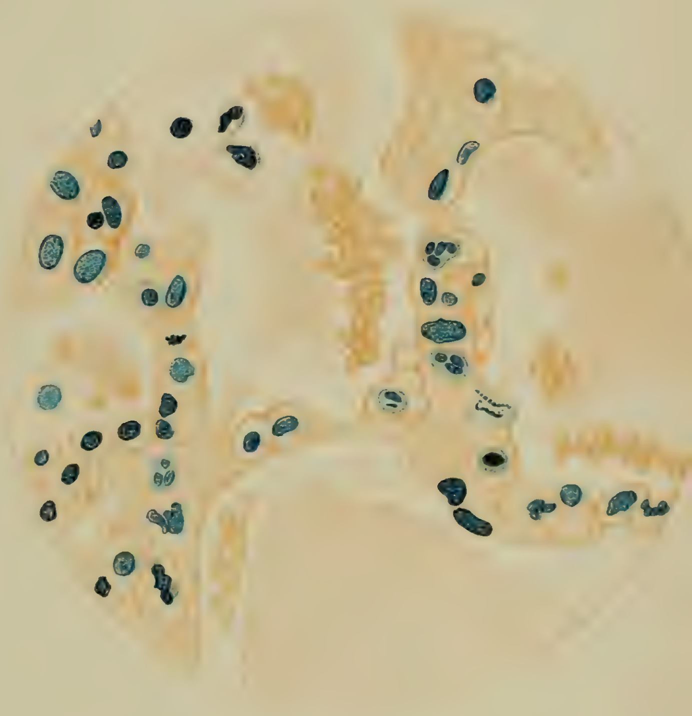

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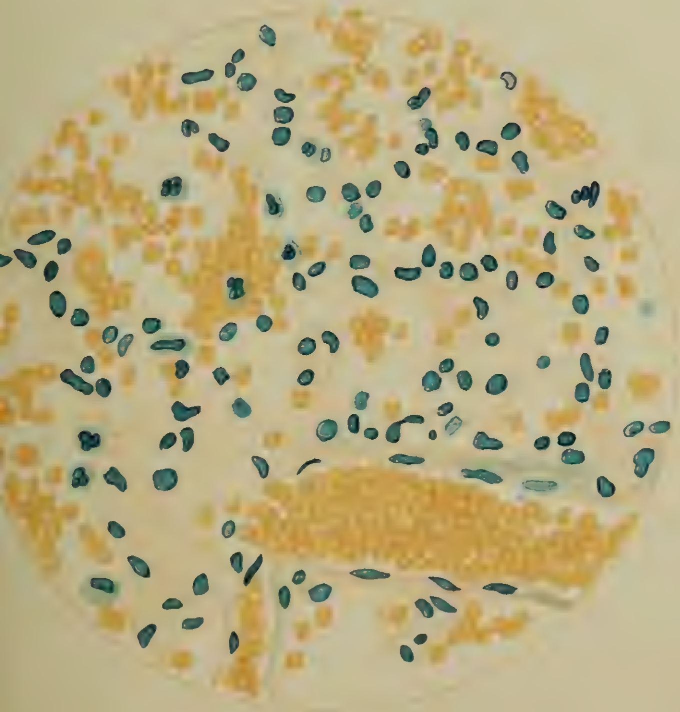

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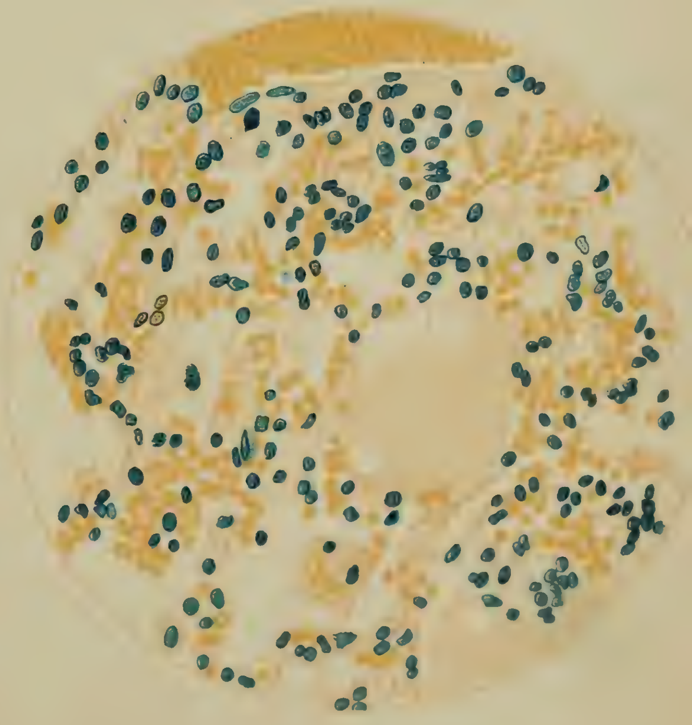

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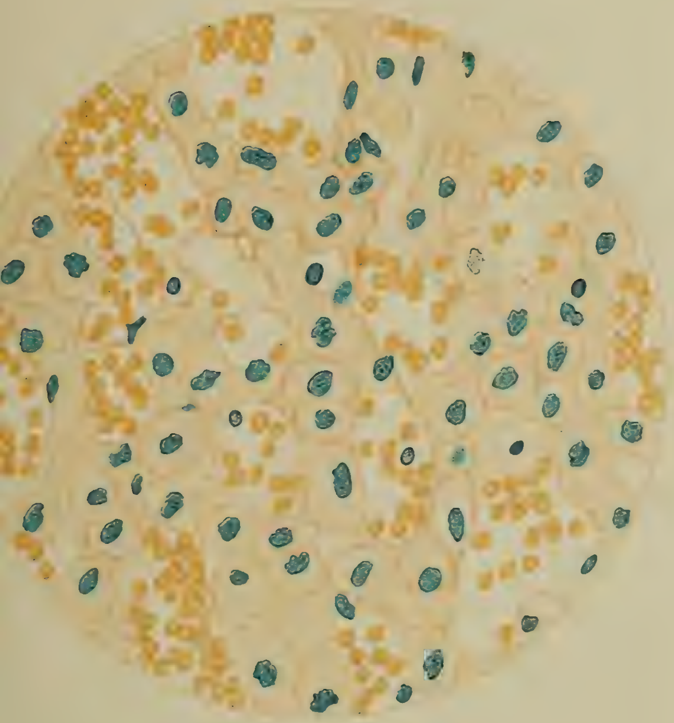

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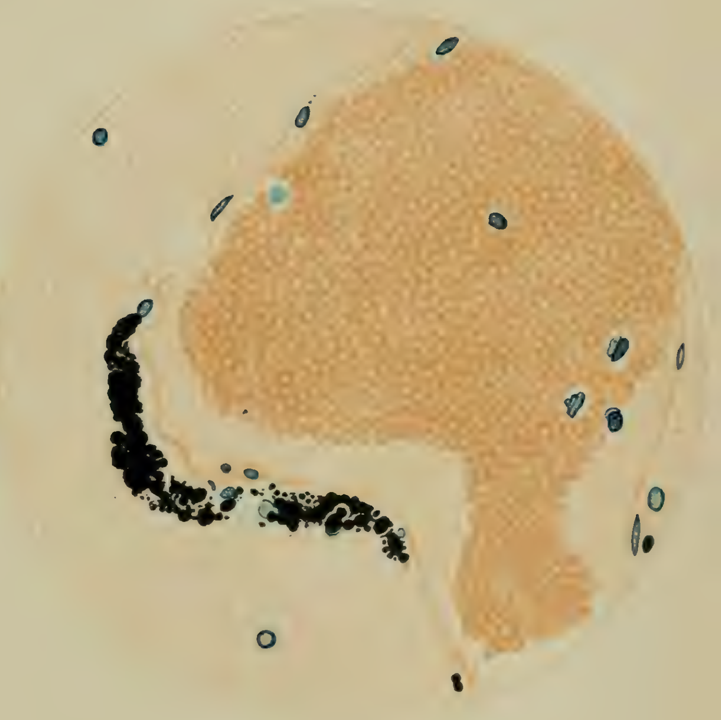

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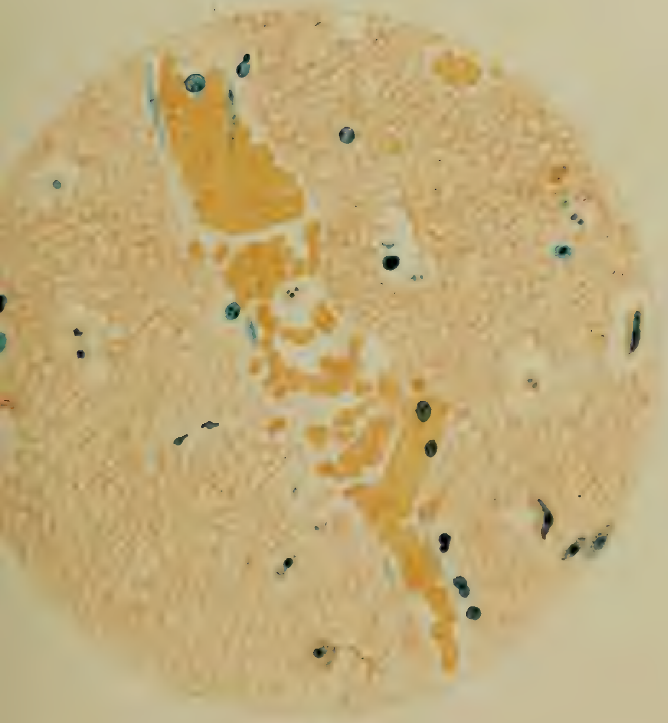

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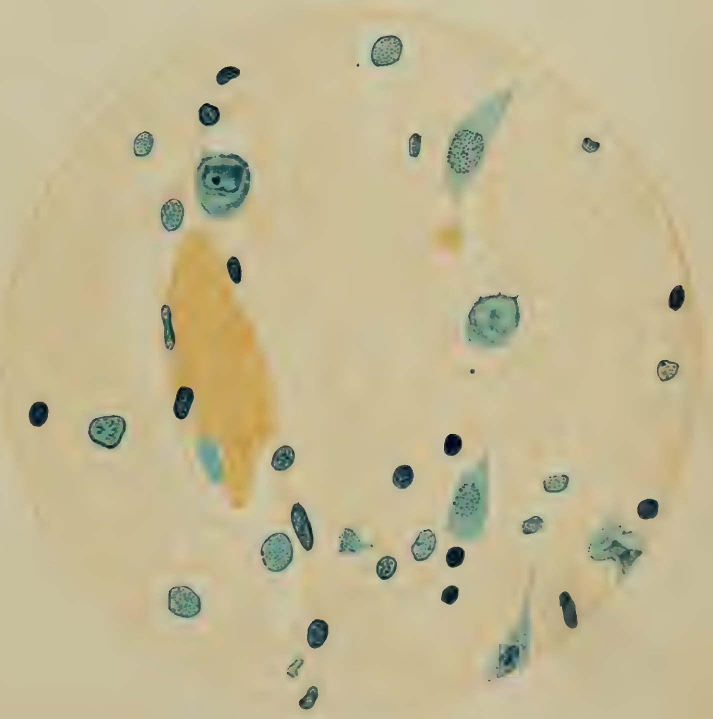

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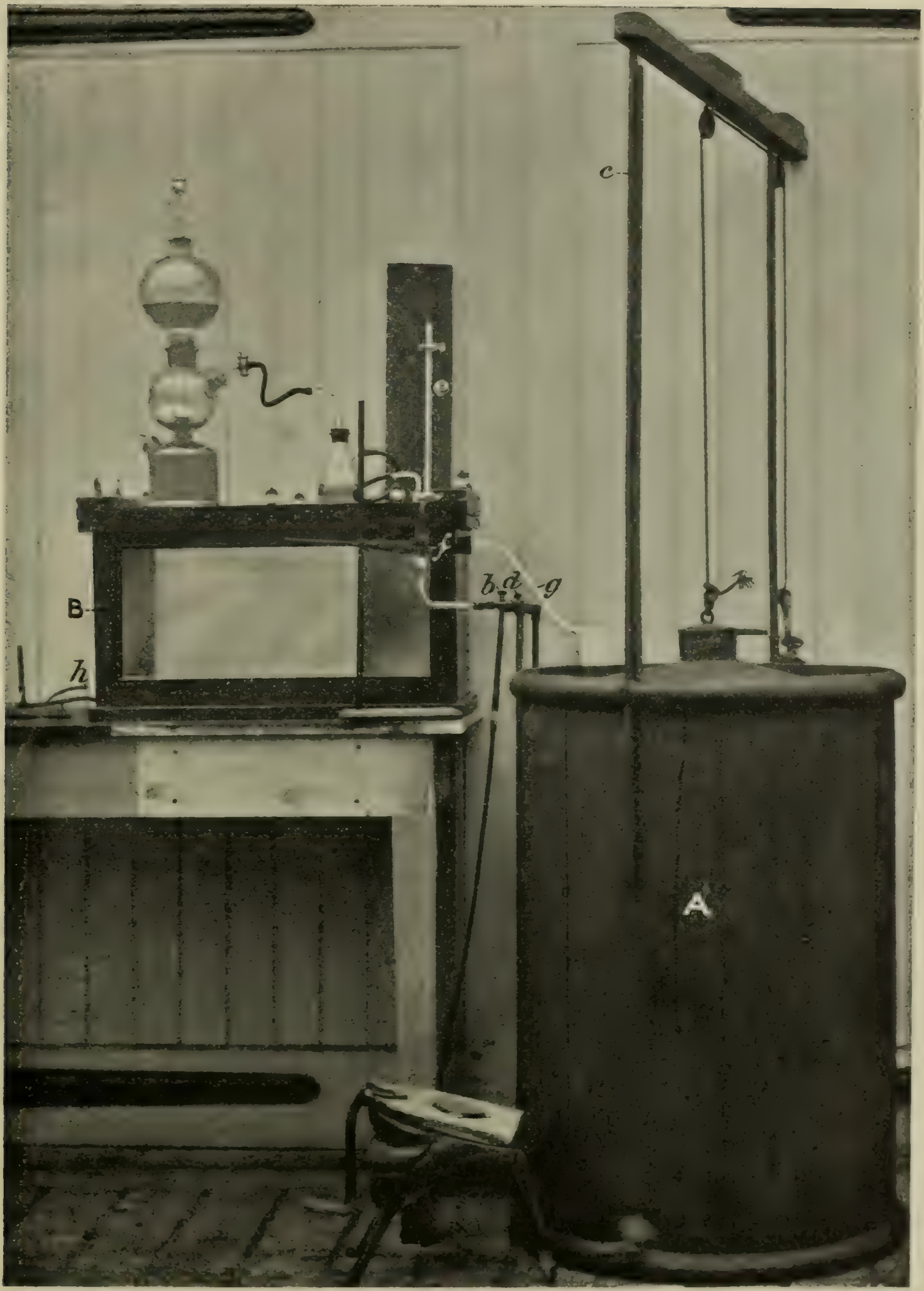

Apparatus used for Experiments
on Nickel Carbonyl.

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BACILLUS DYSENTERIAE OF FLEXNER IN RELATION TO ASYLUM DYSENTERY.

BY H. T. S. AVELINE, A. E. BOYCOTT AND W. F. MACDONALD.

(*From the Lister Institute of Preventive Medicine.*)

IN this country observers are familiar with dysentery as occurring on the one hand in asylums, where it is common [see Mott (1905), Knobel (1906), Candler (1907)], and on the other as scattered sporadic cases (often known as "ulcerative colitis") among the general population with an occasional small epidemic. The bacteriology of these conditions does not appear to have yet been placed upon an altogether satisfactory footing. Of the sporadic English cases little seems to be known beyond the fact that Foulerton (1902) found that the serum of several cases agglutinated Shiga's bacillus. In the case recorded by Saundby and Hewetson (1906) no evidence is adduced that the organism isolated was a dysentery bacillus at all. There is however clear evidence that the institutional disease is associated with *B. dysenteriae.* Hewlett (1904) obtained positive agglutination results with Flexner's bacillus in two cases which were negative with the Shiga type. Eyre (1904) in an important research examined nine acute cases from a London County asylum and isolated Shiga's bacillus from the faeces of 4 out of 5 examined during life, and from 2 out of 4 examined only post-mortem. He further showed that the patients' serum agglutinated Shiga's bacillus while it was without action upon the Flexner organism. Dr Eyre has been good enough to inform us that he met with Flexner's bacillus in 6 of the 9 acute and in 15 of 35 chronic cases. The chronic cases in no instance yielded Shiga's bacillus, and their serum did not agglutinate Shiga or Flexner, which latter organism at the time he regarded as of doubtful import. Finally, McWeeney (1906) isolated from a case of acute fatal asylum dysentery in Ireland an organism which appears to be the *B. dysenteriae* of Flexner though it is stated to have produced no indol and to have been pathogenic for rabbits. The work of Morgan (1906, 1907) has shown that no sort of *B. dysenteriae* is responsible for the summer diarrhoea of children in London as is the case in America and elsewhere.

The present investigations have been carried out on material derived from patients in the Somerset and Bath Asylum at Cotford, near Taunton. The material was of necessity not quite fresh when received in London, but the results indicate that dysentery stools may be subjected to a satisfactory bacteriological examination when they have been passed for fully 24 hours.

In all cases surface plates of MacConkey's bile-salt-lactose-neutral-red agar were used for the separation of the organisms. It is more satisfactory to plate directly when the stool is mucous in character, or from a fresh emulsion when faecal, than after a preliminary incubation in a fluid medium. It was for example found in one of the early cases that plates made directly from the stool showed colonies of *B. dysenteriae* and of *B. coli* in the proportion of 100 to 16, while plates made from an 18 hours culture of the same stool in glucose-bile-salt peptone-water or in ordinary broth showed ten times as many *B. coli* as *B. dysenteriae*. From such plates the non-lactose-fermenting colonies were picked off and tested by cultural and agglutination methods. The colonies of *B. dysenteriae* on MacConkey's agar are to some extent characteristic in that each white colony is surrounded by a clear zone distinctly more yellow (i.e. more alkaline) in colour than the rest of the medium. Of the cultural tests most use was made of the fermentations of various sugars and alcohols, of the reaction in litmus-milk and of the production of indol. Practically, however, it is most convenient to make broth cultures of any suspicious colonies, examine for motility after four to six hours, and, if found non-motile, immediately apply the agglutination test. In this way a reasonably certain diagnosis may be arrived at in 24 hours, and afterwards confirmed by culture.

The serum used for agglutination in these cases was a horse serum prepared for therapeutic use with a number of strains of dysentery bacilli. These included the types of Shiga, Kruse and Flexner, those isolated by Eyre from asylum dysentery and several from cases of infantile diarrhoea in America. It agglutinated the homologous organisms of the Shiga and Flexner types up to a dilution of 1 : 10,000 and was generally used diluted 1 : 1000, the observations being made microscopically at room temperature; in all cases a positive result was reached in two hours if at all.

Clinically the cases examined on the whole conformed to the usual type of acute asylum dysentery. As a rule the onset was sudden with headache, pains in the back and limbs and malaise ending in vomiting: then a rigor or an abrupt rise of temperature to 102°—104° F. Diarrhoea then begins: at first the stools appear normal, then loose and generally after 24 hours small containing blood and slime. Epigastric pain is complained of during the first few days, later pains in the region of the large intestine aggravated by palpation. The stools have a characteristic sour odour and contain greyish-white, tenacious slime intimately mixed with blood: six or eight motions in 24 hours is about the average for the first few days. The average duration under treatment was about 8 days, the temperature reaching normal about the third day, after which the number of stools diminishes. Slime persists in the stools after blood has ceased to be passed.

Working in this way, material from 44 inmates of the asylum was examined: 20 of them had dysentery at the time. The results may be grouped as follows:

(*a*) Of the cases of clinical dysentery, the stools were examined in 19 and *B. dysenteriae* of the Flexner type was in all isolated from 17. In one fatal case the spleen and mesenteric glands were alone available: *B. dysenteriae* was found in both. Three of the positive cases died: in one *B. dysenteriae* was isolated from the stools 14 days before, and also on the day of death, but was not found in the ulcerated coecum post-mortem; in the second it was found in the stools four days before death and also recovered from scrapings of the mucosa of the coecum; in the third death occurred 2½ months after dysentery bacilli had been found in the stools and they were not recovered from the spleen. The stools of five of the positive cases were examined during convalescence: one was negative 13 and 28 days after the attack, one negative after 8 and 30 days, one negative 14 days after, and two negative 25 days later. One case was examined as a normal control two days before the onset of symptoms with a negative result; on the third day of illness *B. dysenteriae* was found in large numbers. Two others had also been previously examined with negative results two and three months respectively before the onset of acute illness. In three acute cases the stools failed to yield dysentery bacilli: in one however these were found 28 days later during convalescence but not 41 days after the acute attack. In another case the symptoms of dysentery continued for 4½ months; the stools were examined three times and the spleen and mesenteric glands post-mortem—all with negative results.

(*b*) The stools were examined in 26 cases which were not clinically dysentery though five of them had diarrhoea. Twelve had had previous attacks of dysentery, in two 5 weeks before examination, in four

2 months, in two 3 months and in three 12 to 14 months. In one case scrapings from small ulcers found in the upper third of the rectum of a patient dying of nervous disease, who had had diarrhoea for three months before death, were examined. In none of these 27 cases was *B. dysenteriae* found. Most of them were contacts in the same wards with cases of dysentery which had been verified bacteriologically. There were for instance at one time three cases of dysentery so verified in one ward; while these persons were ill, stools from ten other patients in the same ward were examined: the results were all negative though three of them subsequently developed clinical dysentery and *B. dysenteriae* was found in their faeces.

The organisms isolated conformed in all respects to the *B. dysenteriae* of the Flexner type[1]. They fermented dextrose, laevulose, galactose and arabinose with the production of acid without gas, did not change the reaction in lactose, dulcite, erythrite, salicin or inulin, caused an initial acidity with a terminal alkalinity in litmus-milk, produced a slight to a moderate amount of indole (paradimethylamidobenzaldehyde test), were not motile and agglutinated with the multivalent anti-dysentery serum up to 1 : 1000 and, when so examined, up to 1 : 10,000. In no instance were any bacilli of the Shiga type found, and a few agglutination tests with the blood of the patients showed no reaction with Shiga's original strain or with Eyre's cultures, while with the bacilli isolated from the corresponding and other cases and with Flexner's strain a good reaction up to a dilution of 1 : 200 or 1 : 500 was sometimes obtained.

In the differentiation of dysentery bacilli further than into the broad divisions of Shiga type (not fermenting mannite) and Flexner type (fermenting mannite), some importance has been attached by Hiss and others to the reactions in media containing maltose and cane-sugar. These authors note that to some extent the reactions are inconstant, especially as to the time at which the acid reaction may appear. The following tests were made simultaneously on 24 cultures from seven cases of the present series. The test sugars were in 1 % solution in a mixture of one part peptone beef broth with three parts peptone-water; incubation was at 36° C. The number of cultures which were acid at different times was as follows:

	Cane-sugar				Maltose			
Days incubated	1	7	14	28	1	7	14	28
Number of cultures acid	0	0	1	4	2	3	13	24

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[1] See the papers of Hiss (1904), Torrey (1905), Shiga (1906) and Dopter (1907).

There can be little doubt that all these cultures must be regarded as being essentially the same bacillus. Even if that were not the case, it is difficult to believe that all the cultures from any one case are not the same. Yet the variations of fermentative power in the whole series of cultures do not in any way correspond to the distribution of the cultures among the cases. Thus the four cultures which eventually produced an acid reaction in the cane-sugar medium came from four different cases, and the three which fermented maltose in seven days also from three different cases.

Summary.

1. *B. dysenteriae* of Flexner has been found in the stools of 17 out of 19 cases of asylum dysentery; no evidence of the presence of the Shiga type was obtained.

2. In 18 cases examined one week to 14 months after an attack of dysentery, *B. dysenteriae* was found only once (3 weeks).

3. No evidence has been obtained of the presence of *B. dysenteriae* in the faeces of ward contacts (26 cases) with either normal or diarrhoeic stools.

4. The fermentative reactions of *B. dysenteriae* of Flexner towards maltose and cane-sugar are variable.

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THE PREVENTION OF COMPRESSED-AIR ILLNESS.

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(From the Lister Institute of Preventive Medicine.)

[With 7 Figures and 3 Plates.]

CONTENTS.

PAGE
Introduction 343
Part I. Theoretical.
  A. The rate of saturation of the body with nitrogen during exposure to compressed air 345
  B. The rate of desaturation of the body with nitrogen during and after decompression 350
  C. The limits of safety in decompression 355
  D. Practical measures for avoiding compressed-air illness:
    (1) Diving 358
    (2) Work in caissons and tunnels 372
Part II. Experimental.
  1. Apparatus 377
  2. Choice of experimental animals and results with large and small animals 378
  3. Respiratory exchange of goats 380
  4. Method of conducting the experiments 382
  5. Symptoms observed in goats 385
  6. Results of the goat experiments 394
  7. Individual variation among the experimental animals . . . 403
  8. Pathology of caisson disease in goats 410
Summary 424
Appendix I. Human experiments in the pressure chamber 426
  ,, II. Human experiments in deep diving 429
  ,, III. Protocols of some goat experiments 436
  ,, IV. Tables for decompression of divers 442

INTRODUCTION.

MEN who have been working in compressed air, as in diving, preparing foundations of bridges, etc. under water, or making tunnels or shafts through water-bearing strata, are liable on their return to atmospheric pressure to a variety of symptoms generally known as "diver's palsy" or "caisson disease," but which may more conveniently be designated "compressed-air illness." It was shown experimentally by Paul Bert[1] that these symptoms are due to the fact that gas (chiefly nitrogen) which goes into solution in the blood and tissues during exposure to compressed air is liberated in the form of bubbles on too rapid decompression, and produces local or general blockage of the circulation or other injury. Subsequent investigations, for an account of which we must refer more particularly to the treatise on the subject by Heller, Mager and v. Schrötter[2] and to recent papers by Hill and McLeod[3] and Hill and Greenwood[4], have confirmed and extended Paul Bert's conclusions.

It was pointed out by Paul Bert that by means of very slow decompression the symptoms of caisson disease could be avoided, but his experiments were not sufficient to furnish data as to what rate of decompression would be safe. Nor has subsequent human experience in engineering undertakings solved this problem; and the risks attending work in compressed air at excess pressures of over 1½ to 2 atmospheres are notorious. Heller, Mager and v. Schrötter have endeavoured to formulate rules as to safe decompression; and they express the belief that perfectly uniform decompression at the rate of 20 minutes an atmosphere would always be safe. Following this rule, which is based on a calculation, Hill and Greenwood decompressed themselves, without any serious symptoms, after short exposures at excess pressures of as much as five and even six atmospheres.

Although the rules formulated by the above-mentioned observers constituted a distinct step in advance, it appeared to us that, for reasons which will be explained below, there were grave doubts as to the safety of their recommendations, and particularly as to whether uniform decompression is desirable. The need for framing definite rules as to safe decompression in the shortest possible time presented itself in a very definite form in connection with the work of the Admiralty Committee on Deep Diving[1], of which one of us was a member. Our investigation, which was planned with the more particular object of furnishing information required for securing the safety of divers ascending from deep water, was rendered possible by the gift to the Lister Institute by Dr Ludwig Mond, F.R.S. of a large experimental steel pressure chamber and by substantial financial and other help from the Admiralty, Messrs John Aird and Son, the late Mr Basil Ellis, Messrs S. Pearson and Son, Ltd., and Messrs Price and Reeves.

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[1] *La Pression Barometrique*, 1878.

[2] *Luftdruckerkrankungen*, 1900; also v. Schrötter, *Der Sauerstoff in der Prophylaxie und Therapie der Luftdruckerkrankungen*, 2nd edition, 1906. The former work contains a very full abstract of all previous investigations on the subject.

[3] This *Journal*, vol. III. (1903), p. 401 (and references there given): see also *Recent Advances in Physiology*, 1906, pp. 233—255.

[4] *Proceedings of the Royal Society*, vol. LXXVII. p. 442, 1906; vol. LXXIX. p. 21, 1907; also *British Medical Journal*, July 7th, 1906, Feb. 16th, 1907, June 22nd, 1907.

The formation of gas bubbles in the living body during or shortly after decompression evidently depends on the fact that the partial pressure of the gas or gases dissolved in the blood and tissues is in excess of the external pressure. But it is a well-known fact that liquids, and especially albuminous liquids such as blood, will hold gas for long periods in a state of supersaturation, provided the supersaturation does not exceed a certain limit. In order to decompress safely it is evidently necessary to prevent this limit being exceeded before the end of decompression. Whether or not the decompression is free from risk will depend on the degree of supersaturation which can be borne with safety, the extent to which the blood and tissues have had time or opportunity to become saturated, and the extent to which they have had time to become desaturated again during decompression. In carrying out our investigations we have kept these three factors constantly in view, and it is necessary to discuss them in some detail before proceeding further.

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[1] The Report of this Committee, which has recently appeared as a blue-book, contains a full account of the experimental investigations on Diving, carried out under its auspices at Portsmouth, off the West Coast of Scotland, and elsewhere, during the last two years: also a short summary of the experiments detailed in the present paper, and many data as to the occurrence of compressed-air illness in connection with diving and other work in compressed air. The conclusions and recommendations of the Committee are summarised at the beginning of the Report.

PART I. THEORETICAL.

A. *The rate of saturation of the body with nitrogen during exposure to compressed air.*

When a man or animal is placed in compressed air, the blood passing through the lungs will undoubtedly take up in simple solution an amount of gas which will be increased above normal in proportion to the increase in partial pressure of each gas present in the alveolar air. The experiments of Haldane and Priestley[1], which have since been extended by Hill and Greenwood[2], show that the partial pressure of CO_2 in the alveolar air remains constant with a rise of atmospheric pressure: hence there can be no increase in the amount of CO_2 present in the blood during exposure to compressed air. As regards oxygen, the amount in simple solution in the arterial blood will certainly increase in proportion to the rise in alveolar oxygen pressure; but as soon as the blood reaches the tissues this extra dissolved oxygen, which (except with exposures to enormous pressures) is only a small part of the total available oxygen in the arterial blood, will be used up, so that in the tissues and venous blood there will be at most only a very slight increase in the partial pressure of oxygen. For practical purposes therefore we need only take into consideration the saturation of the body with nitrogen.

In view of what is known as to the ease and completeness with which the blood becomes aerated in its passage through the lungs, there seems no reason to doubt that in compressed air the blood reaching the lung capillaries must become instantly saturated with nitrogen at the partial pressure existing in the alveolar air (see p. 351). At the commencement of exposure to compressed air this blood, on being carried to the tissues, will by diffusion share with them its excess of nitrogen and then return to the lungs for a fresh charge. By the constant repetition of this process the tissues, and the venous blood leaving them, will gradually become more and more saturated with nitrogen at the partial pressure of the nitrogen in the alveolar air, which will be practically the same as in the inspired air. Since the rate of blood supply and the solubility of nitrogen per unit mass of tissue vary greatly in different parts of the body, the rate of saturation will vary correspondingly. We may however form some rough general idea of the average rate of saturation by assuming as a basis of calculation that the blood is evenly distributed throughout the body, and that the tissues are similarly constituted in all parts.

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[1] *Journal of Physiology*, vol. XXXII. (1905), p. 229.

[2] *Proc. Roy. Soc.*, B, vol. LXXVII. p. 442.

According to the figures adopted by Bohr[1], 100 c.c. of blood take up in simple solution at the body temperature 0·87 c.c. of nitrogen for each atmosphere of air pressure. This is only 8 % less than would be taken up by water under the same conditions. Blood contains nearly the same percentage of solids as the semi-liquid tissues (apart from fat) in most parts of the body, and we may assume that these tissues will take up nearly the same proportion of nitrogen as blood. The earthy constituents of bone (about 3 % of the body weight) probably take up no nitrogen. On the other hand the body fat, as was recently shown by Vernon[2], who made a number of determinations at the body temperature with special reference to our investigations, takes up about six times as much nitrogen as an equal weight of blood. The body of a well-nourished man probably contains fully 15 % of its weight as fat or fatty material. Hence it may be estimated that it will, when saturated at any given pressure, on an average take up, weight for weight, about 70 % more nitrogen in simple solution than the blood under the same conditions, and that the whole body of a man weighing 70 kilos will take up about one litre of nitrogen for each atmosphere of excess pressure.

Now the weight of the blood in man is about 4·9 % of the body weight[3]: hence the amount of nitrogen held in solution in the body, when it is completely saturated at any given pressure, will be about $\frac{170}{4 \cdot 9}$, or 35 times as great as the amount present in the blood alone. If therefore the blood distributed itself evenly and at the same rate throughout the body, the latter would have received, at the end of one complete round of the blood after sudden exposure to high pressure of air, one thirty-fifth of the excess of nitrogen corresponding to complete saturation. The second round of the circulation would add one thirty-fifth of the remaining deficit in saturation, *i.e.* $1/35 \times 34/35$ of the total excess : the third round would add $1/35 \times (34/35 \times 34/35)$ of the total excess, and so on. On following out this calculation, it will be found that half the total excess of nitrogen would have entered the body after 23 rounds of the circulation, three-fourths after 46 rounds, seven-eighths after 69 rounds, and so on. The progress of the saturation of the body with nitrogen is thus a logarithmic curve of the form shown in Figure 1[1].

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[1] *Nagel's Handbuch der Physiologie*, vol. I., 1905, p. 63.

[2] *Proc. Roy. Soc.*, vol. LXXIX. B, 1907, p. 366.

[3] Haldane and Lorrain Smith, *Journal of Physiology*, vol. XXV., 1900, p. 340.

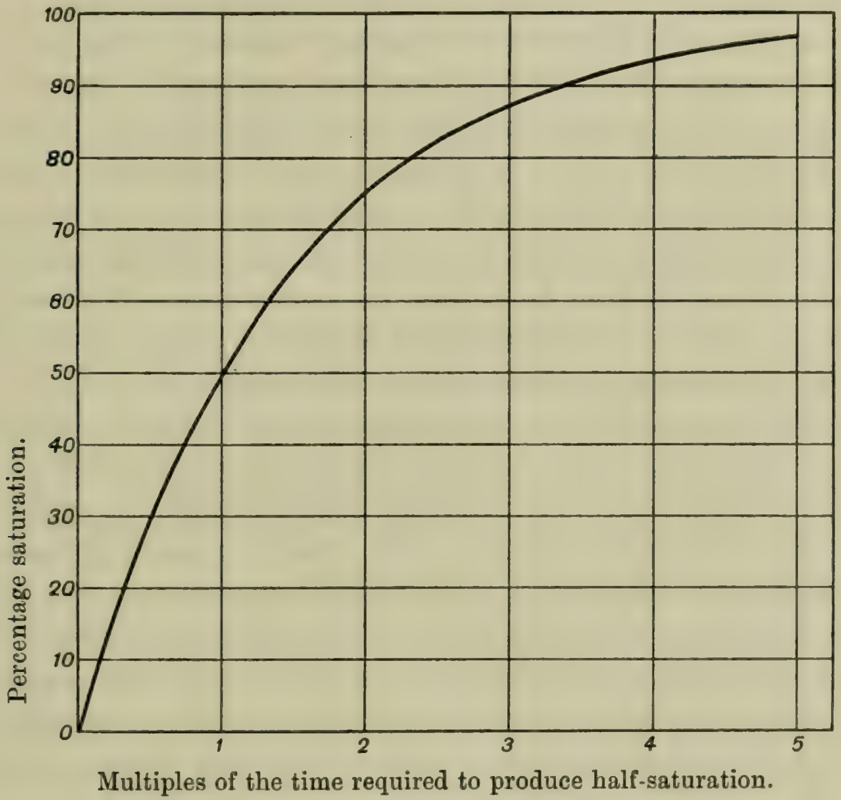

Fig. 1. Curve showing the progress of saturation of any part of the body with nitrogen after any given sudden rise of air pressure. The percentage saturation can be read off on the curve, provided the duration of exposure to the pressure, and the time required to produce half-saturation of the part in question, are both known. Thus a part which half-saturates in one hour would, as shown on the curve, be 30 °/₀ saturated in half-an-hour, or 94 °/₀ saturated in 4 hours.

Experiments on animals have shown that the venous blood entering the lungs contains about two-fifths less of oxygen than the arterial blood. If we assume that the same proportion holds good for a man at rest, and that very little oxygen is used up in the lungs themselves, the percentage of oxygen gained by the blood in the lungs must be about 8 °/₀, or about double the percentage diminution in the expired air as compared with the inspired air. The volume of blood passing through the lungs is therefore about double the volume of air breathed. Since this volume of air (measured dry and at standard pressure and temperature) averages about seven litres per minute[1] for a man of 70 kilos during rest, the volume of blood passing through the lungs may be estimated at about 3·5 litres per minute[2]. The total blood volume is however also about 3·5 litres, so that a volume of blood equal to the total blood volume probably passes through the lungs about once a minute during rest. We may therefore substitute minutes for rounds of the circulation in the above calculation of the rate of saturation of the body with nitrogen, so that, if the assumptions made for the purposes of the calculation held good for a man exposed to compressed air, his body would be half saturated with the excess of nitrogen in 23 minutes, three-fourths saturated in 46 minutes, etc.

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[1] This calculation is in principle similar to that made by Zuntz (*Fortschritte der Medizin*, 1897, No. 16), and worked out more fully by Heller, Mager and v. Schrötter (*loc. cit.*). On account, however, of the discovery that fat has a very high coefficient of absorption for nitrogen, and that the blood volume in man is considerably less than was formerly supposed, our calculation gives a much slower rate of saturation per round of the circulation.

In reality, however, this calculation affords at best only a very rough general idea of the actual rate of saturation, since it is known that the distribution of blood per unit of body weight through various parts of the body varies greatly, and that the rate of circulation through any given part varies according as the part is at rest or in a state of activity. The proportion of fat and fatty material is also very different in different parts of the body, so that the capacities of different tissues for taking up nitrogen must vary accordingly. We should expect therefore that some parts of the body will saturate much more rapidly than the calculation shows, and other parts much more slowly. Direct experimental evidence of far more rapid saturation in some parts of the body has recently been furnished by Hill and Greenwood[3]. Their method was to determine the free nitrogen in samples of urine secreted shortly after exposure to high pressure, and shortly after return to normal pressure. A sufficiently copious secretion of urine was produced by previously administering large drinks of water to the subject of the experiment; and they found that, within about ten minutes of exposure to high pressure, samples of urine secreted were saturated at this pressure. Conversely, on lowering the pressure to normal, the excess of nitrogen disappeared within a few minutes.

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[1] Haldane and Priestley, *loc. cit.*, p. 245.

[2] As a result of numerous experiments on man with the lung catheter Loewy and v. Schrötter (*Untersuchungen über die Blutcirculation beim Menschen*, 1905, p. 90) infer that the average rate of blood flow during rest is slightly faster. At present, however, there is some doubt as to the interpretation of results obtained by the lung catheter method.

[3] *Proc. Roy. Soc.* B, vol. LXXIX., p. 21, 1907.

These results seem to show conclusively that the kidney substance became saturated with nitrogen at a rate about ten times as great as would correspond to the above calculation. From the data given it appears, however, that urine was being secreted with great rapidity during the experiments. For instance, 135 c.c. were secreted in five minutes in one observation where the quantities and times are recorded. This is about thirty times the average rate of secretion, so that the circulation of blood through the actively working kidneys must have been greatly increased.

Equally clear evidence of the existence of a far slower rate of saturation is afforded by the experience of men working in compressed air, particularly in caissons and tunnels at moderate pressures. It is well known to those practically familiar with such work that the risk of symptoms occurring on decompression depends on the duration of the exposure. There is very little risk on rapid decompression after short exposures of less than an hour to an excess pressure of two atmospheres or even somewhat higher pressure; but as the duration of exposure increases hour by hour, so do the risks on decompression increase. We are assured by Mr E. W. Moir (of the firm of Messrs S. Pearson and Son, Ltd., Westminster), who has had an exceptionally large experience of tunnelling work in compressed air at excess pressures up to about 2½ atmospheres, that the maximum of risk is not reached after even three hours, so that a limitation of working shifts to three hours markedly diminishes the frequency of compressed-air illness. Hence in some parts of the body saturation with nitrogen must still be incomplete after three hours. Another observation pointing in the same direction is that when the daily working period was 8½ hours under pressure with two intervals of about 1½ hours each for meals at ordinary atmospheric pressure, cases of caisson disease usually occurred after the last decompression in the evening and not when the men came out for meals[1].

Our own observations on animals afford fresh evidence bearing in the same direction. We found that in goats the risks on decompression increase with the length of exposure to pressure up to from two to three hours (see below, p. 396).

In different warm-blooded animals the rate of respiratory exchange varies, roughly speaking, according to the ratio of body surface to weight. The smaller the animals, therefore, the greater is the respiratory exchange per unit of body weight, and the more rapid must be the circulation. In consequence small animals, when placed in compressed air, must saturate their tissues more rapidly in proportion to their more active respiratory exchange; and, conversely, they will free themselves more rapidly, during or after decompression, from the excess of nitrogen. Hence results obtained with small animals as to the time required for complete saturation, or for safe decompression, are not directly applicable to man. We selected goats for our experiments as they were the largest animals which could be conveniently used; but their weights averaged only about one-fourth to one-third of the weight of an adult man. As the surfaces of different mammals are roughly as the cube roots squared of their weights, we should expect that in goats of this size the respiratory exchange per kilo of body weight would be about two-thirds greater than in man. Direct determinations showed that this was the case (see p. 381). Hence if it required three hours exposure to a high pressure to effect practically complete saturation[1] of the more slowly saturating tissues of a goat with nitrogen, about five hours would be required for a man. An inspection of Fig. 1 (p. 347) will show that if these tissues became 50 % saturated in about 45 minutes in goats and 75 minutes in man, they would be 94 % saturated in three hours for goats, and in five hours for man. A higher degree of saturation than this would scarcely be appreciable, and we have concluded that for practical purposes any slower rate of saturation than this, and correspondingly slower rate of desaturation, need not be allowed for, unless the percentage of fat in the body is abnormally high. We must admit, however, that there is some evidence, both from our own experiments and from practical experience in work in compressed air, that in the parts of the body which are the seat of "bends" a still slower rate of saturation may exist.

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[1] G. W. M. Boycott, *Trans. Inst. of Civil Engineers*, vol. CLXV., 1906.

B. *The rate of desaturation of the body with nitrogen during and after decompression.*

If the pressure is rapidly diminished to normal after exposure to saturation in compressed air, and no gas bubbles are liberated in the body, it is evident that for each part of the body the curve of desaturation will be similar to that of saturation, provided the physiological conditions are constant. The venous blood will give off practically the whole of its excess of dissolved nitrogen during its passage through the lungs[1], and at each round of the circulation will bring back a fresh charge of nitrogen (at the partial pressure existing in the tissues) to be given off. The parts which become half desaturated by this process in a given time will be three-fourths desaturated in double the time, and so on. The slowest saturating tissues will thus, in accordance with our previous calculation, take one and a quarter hours to become half desaturated in man.

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[1] The only method apparently available to determine the time of complete saturation in normal animals is to subject them to a series of experiments in which the pressure and decompression are kept constant and the time of exposure varied, and to observe when the effects cease to become any worse. The method is open to obvious limitations.

The normal combined gas pressure of nitrogen, oxygen and CO_2 in the tissues and venous blood may be estimated as about 90 % of an atmosphere, so that if the nitrogen pressure be more than an eighth above normal the total gas pressure will be above atmospheric pressure. Supposing therefore that before decompression the most slowly saturating parts of the body (*i.e.* those half saturating in one and a quarter hours) had been saturated to an excess pressure of two atmospheres of air, it would take about five hours at atmospheric pressure to reduce this excess pressure to a sixteenth (or an eighth of one atmosphere) and so bring down the total gas pressure in the parts in question to about atmospheric pressure. The slowness of desaturation must be as clearly borne in mind as the slowness of saturation, in connection with all the phenomena of compressed-air illness.

If gas bubbles are formed in consequence of too rapid decompression, they will naturally tend to increase in size by diffusion into them, in whatever part of the body they may be except the arteries, for some time after the end of decompression. They may thus easily cause blocking of small vessels, and even if they are carried to the right side of the heart or the pulmonary arteries, and lodge there, they will increase in bulk until the total gas pressure in the mixed venous blood falls to one atmosphere. The same remark applies to bubbles which lodge in the branches of the portal veins. If small bubbles are carried through the lung capillaries and pass, for instance, to a slowly desaturating part of the spinal cord, they will there increase in size and may produce serious blockage of the circulation or direct mechanical damage. Apart from this increase of size the air bubbles passing along the arteries are probably too small to cause any harm. Once formed they will under ordinary conditions take a long time to become reabsorbed, since even after the gas pressure in the blood and tissues has fallen to normal, the excess of nitrogen pressure in the bubbles over that in the blood and tissues will only be about a tenth of an atmosphere at most. In one case we found bubbles in the veins of an animal which died two days after suffering from severe decompression symptoms (see below p. 421).

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[1] In view of the enormous surface (probably more than 100 square metres) presented by the lung alveoli for diffusion it seems hardly possible to doubt that the blood during its passage through the lungs becomes saturated or desaturated to almost exactly the pressure of nitrogen in the alveolar air. According to the calculations of Loewy and Zuntz (*Die physiologischen Grundlagen der Sauerstoff-Therapie* in Michaelis' *Die Sauerstofftherapie*, Berlin, 1904), a difference in partial pressure of oxygen of less than 1 mm. of mercury would account for the diffusion of 250 c.c. of oxygen per minute through the alveolar walls. With a difference in partial pressure of nitrogen of two atmospheres, or 1520 mm. of mercury, between the blood and the alveolar air only about 70 c.c. of nitrogen would require to pass per minute in order to establish complete saturation, or desaturation, of the blood. The conditions are thus enormously more favourable for the taking up or giving off of this nitrogen than for the taking up of oxygen by diffusion during normal respiration.

In order to avoid the risk of bubbles being formed on decompression, it has hitherto been recommended that decompression should be slow and at as nearly a uniform rate throughout as possible. We must therefore carefully consider the process of desaturation of the body during slow and uniform decompression. For convenience in calculation we may imagine the process as occurring in a series of time-intervals, the first half of each of which is spent at the pressure existing at the beginning of the interval, and the second half at the pressure existing at the end. Let us suppose, for instance, that the body has been completely saturated with nitrogen at an excess pressure of five atmospheres of air, and that decompression occurs at a rate of one atmosphere in 20 minutes. The process may be divided into five periods of 20 minutes, during each of which the pressure falls one atmosphere. We can then easily calculate how far desaturation will have gone at the end of each period, and from these data construct a desaturation curve.

Let us first consider the mean desaturation rate of the whole body, assuming that, when the pressure is suddenly raised or diminished to a certain level, the tissues will on an average saturate or desaturate themselves by 50 % in 23 minutes, which was shown above to be a probable average rate. A reference to the curve (Fig. 1) shows that ten minutes' exposure to the reduced pressure of four atmospheres in excess will reduce the saturation by 28 % of the difference between five and four atmospheres, *i.e.* by 0·28 of an atmosphere. Hence at the end of 20 minutes the tissues will on an average be saturated to 4·72 atmospheres. Ten more minutes at four atmospheres will reduce the saturation to 4·5 atmospheres, and ten minutes at three atmospheres will further reduce it by 28 % of 4·5 – 3, *i.e.* by 0·42 atmosphere. Hence at the end of the second twenty minutes the saturation of the tissues will be 4·08 atmospheres. Continuing this calculation we get the desaturation curve shown in Fig. 2, from which it will be seen that when atmospheric pressure is reached the tissues are still saturated to an excess pressure corresponding to 1·4 atmospheres of air.

Fig. 2 also shows a similar curve for the parts which saturate and desaturate most slowly, and which, according to our previous calculations, take one and a quarter hours to become half saturated. At the end of decompression these slowly desaturating parts, as shown on the curve, are still saturated to 3·15 atmospheres. This of course represents a most formidable excess; and, as will be shown below (p. 401), uniform decompression at this rate is dangerous even to goats, and would certainly be extremely dangerous to men, who desaturate a good deal more slowly than goats.

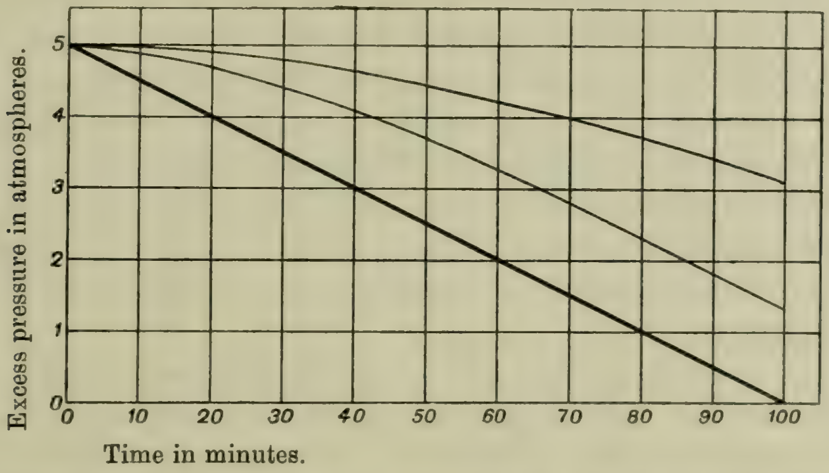

Fig. 2. Desaturation during uniform decompression after complete saturation at 5 atmospheres excess pressure. The thick line represents the air pressure: the upper and lower thin lines represent respectively the progress of desaturation in parts of the body which half saturate in 75 and 23 minutes.

Inspection of Fig. 2 shows that with uniform decompression the nitrogen pressure in the body lags behind that of the air, and that (in the case of the slowly desaturating parts) the amount of the lag increases during the whole time of a decompression lasting 100 minutes. No other result seems possible, and actual experiments point strongly in the same direction, as will be shown presently. We must emphatically dissent from the conclusion drawn by Heller, Mager and v. Schrötter that decompression at the uniform rate of 20 minutes an atmosphere prevents any dangerous retention of gas in the body. To prevent a maximum lag of more than one atmosphere, it would be necessary to decompress at a rate of over one and a half hours an atmosphere if the decompression were uniform and from an excess pressure of five atmospheres[1].

The examples given will be sufficient to illustrate the extreme slowness with which desaturation must occur with a uniform rate of decompression. This slowness has never hitherto been recognised, but must evidently be reckoned with in devising measures for the prevention of caisson disease.

It is clear that the rate of desaturation might be hastened by either (1) increasing the difference in nitrogen pressure between the venous blood and the air in the lungs, or (2) increasing the rate of blood circulation. In either case the blood would give off through the lungs an increased amount of the excess of nitrogen in a given time.

In order to increase the difference in nitrogen pressure between the venous blood and the alveolar air it has been proposed to give a diver oxygen to breathe during, or before decompression. As long, however, as the pressure was above about one atmosphere in excess, or 15 lbs., it would be impossible to do this safely, since, as will be explained more fully below, the effects might be rapidly fatal owing to oxygen poisoning. The possible applications of oxygen are thus somewhat limited, while the complications involved would be very considerable. The same end can, however, be attained in another way, as will be shown in the following section.

The rate of blood circulation can be increased considerably by muscular exertion. Quite moderate exertion is sufficient to increase the respiratory exchange to three or four times the normal; and the rate of blood flow through the lungs must be increased to something approaching to a corresponding extent. Unfortunately, the increased blood flow is chiefly through the muscles which are working, but probably many parts of the body participate to a greater or less extent in the extra blood supply. Muscular work must correspondingly increase the rate of saturation of the body with nitrogen. For this reason it seems desirable that where work has been done in compressed air, so that the muscles and associated tissues have probably become rapidly saturated with nitrogen, there should also be muscular exertion during decompression. The rate of desaturation will thus be increased so as to compensate for the increased rate of saturation. In the case of short exposures to compressed air, as in diving work, this is specially important. Even, however, when there has been no special muscular work in the compressed air movements of joints and massage of the skin etc. will probably hasten desaturation. This has been clearly pointed out by Hill and Greenwood[1].

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[1] It is evidently a mistake to assume that a given rate of uniform decompression, such as 20 minutes per atmosphere, is either necessary for safety in all cases, or would be actually safe except from some limit of pressure. From a pressure below this limit the rate will be unnecessarily slow, and from above it dangerously fast.

Another method which can be employed for increasing the circulation in the case of divers is to restrict the air supply, so that the partial pressure of CO_2 in the air of the helmet may rise sufficiently to stimulate the respiration and circulation. Both methods are now used in the Royal Navy during the ascent of divers.

C. *The limits of safety in decompression.*

It is a fact well known to those practically acquainted with work in compressed air that even with very rapid decompression there is no risk of caisson disease unless the pressure has exceeded a certain amount. It seems perfectly clear that no symptoms occur with less than one atmosphere[2] of excess pressure, however long the exposure may be. Whether any distinct symptoms ever occur with less than about 1·25 atmospheres (18½ lbs. per square inch or 41 feet of sea water) seems very doubtful: at any rate they are very exceptional. At pressures a little above 1·25 atmospheres occasional slight cases begin to be observed, and their frequency and gravity rapidly increase with higher pressures unless the time of exposure is limited or slow decompression is resorted to. The lowest pressure at which we have been able to find any record of a death occurring from caisson disease is 23 lbs. or 1·6 atmospheres[3]. As will be seen below, we were able to obtain slight symptoms on rapid decompression in 1 out of 22 goats after long exposure (four hours) to 1·36 atmospheres or 20 lbs. With 25 lbs. (1·7 atmospheres) two cases of slight illness occurred out of 23 animals.

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[1] *Proc. Roy. Soc.* B, vol. LXXVII., p. 449, 1906.

[2] One atmosphere or 760 mm. of mercury = 14·7 lbs. per square inch, about 1 kilogram per square centimetre, 34 feet of fresh water, 33 feet of sea water. In this paper where pressures are defined in pounds or atmospheres without qualification, reference is intended to the excess over atmospheric pressure as shown on gauges, not to the absolute total pressure.

[3] Babington and Cuthbert, *Dublin Quarterly Journal of Medical Sciences*, vol. XXXVI., 1863, p. 312. In the list of fatal cases given by Heller, Mager and v. Schrötter (*Luftdruckerkrankungen*, p. 1072), are entered two deaths at a pressure of 1·4 atmospheres. A perusal of Paul Bert's original account (*La Pression Barometrique*, p. 401) shows that both the pressure and the cause of death are quite uncertain.

If the risks of rapid decompression depended simply on the extent to which the blood and tissues are supersaturated with nitrogen on decompression, we should expect to find that even a short exposure to such an excess pressure as two atmospheres would be risky with rapid decompression: for there can be no doubt that within, say, half an hour or forty minutes the tissues, and the blood returning from them, must be for all practical purposes fully saturated in many parts of the body, and particularly in parts of great physiological importance which are richly supplied with blood. Nevertheless it seems to be well established that a man may stay without serious risk for forty minutes at a pressure which would involve great danger on rapid decompression if he remained in it for several hours.

Parts of the body with a rapid circulation will become very completely saturated in a comparatively short time, but the highly supersaturated blood which first returns from them on rapid decompression can remain but a very short time supersaturated during each round of the circulation, and on reaching the large veins will mix with less highly saturated blood from other parts of the body. It would seem that the state of high supersaturation in any portion of blood lasts for too short a time to enable bubbles to form.

If this interpretation of the facts is correct, we should expect to find with small animals, which rapidly saturate and desaturate, that a higher pressure would be required to produce symptoms on rapid decompression after a long exposure than in the case of larger animals. The general experience of previous observers is in accord with this, and our own experiments (see below p. 402) showed that we could produce no obvious effects in mice, and very few in rabbits, rats, and guinea-pigs, by sudden decompression after exposures at pressures which were invariably or frequently fatal to goats.

Since supersaturation to the extent of about 1·25 atmospheres above normal atmospheric pressure can be borne with impunity, though a greater degree of supersaturation is risky, it seems clear that, in decompressing after prolonged exposure to high pressures, the rate of decompression should be sufficiently slow to prevent any greater excess of saturation than this in any part of the body at the end of decompression. On the other hand decompression should evidently be as rapid as is possible, consistently with safety. A pressure of 1 to 1·25 atmospheres above normal corresponds to from 2 to 2·25 times the normal atmospheric pressure; but the *volume* (not the *mass*) of gas (measured at the existing pressure) which would be liberated if the whole excess of gas present in supersaturation were given off is the same whether the absolute pressure is reduced from two to one atmospheres, or from four to two, or from eight to four. Hence it seemed probable that, if it is safe to decompress suddenly from two atmospheres of absolute pressure to one, it would be equally safe to decompress from four atmospheres absolute to two, from six atmospheres absolute to three, etc. Our experiments, which are detailed below (p. 398), have shown that this is the case[1]. The process of desaturation can therefore be hastened very greatly by rapidly reducing the absolute pressure to half, and so arranging the rest of the decompression that the saturation in no part of the body shall ever be allowed to correspond to more than about double the air pressure. The main advantage of this plan is that the discharge of nitrogen from the tissues is from the outset of decompression increased to the greatest rate which is safe. The rate of discharge evidently depends on the difference in partial pressure of nitrogen between the venous blood and the alveolar air; and by keeping this difference at the maximum consistent with safety a great saving of time is effected. Detailed investigations have completely justified the adoption of this principle: they are described below, and comprise, besides a series of observations on animals, a number of experiments in which Lieut. Damant and Mr Catto were exposed to excess pressures up to 80 pounds, or 6·4 atmospheres of absolute pressure, in the experimental chamber and to 93½ pounds, or 7·4 atmospheres, in actual diving. The method greatly simplifies the problem of safe decompression, and gets rid of many practical difficulties, particularly in connection with deep diving. It may be conveniently referred to as the method of "stage decompression," and is so described in the sequel, though its essential peculiarity does not lie in the decompression being done in stages but in its being rapid till the absolute pressure is halved and slow afterwards.

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[1] Whether the law holds good for pressures much exceeding six atmospheres is still doubtful, as no experimental data exist.

D. *Practical measures for avoiding Compressed-air Illness.*

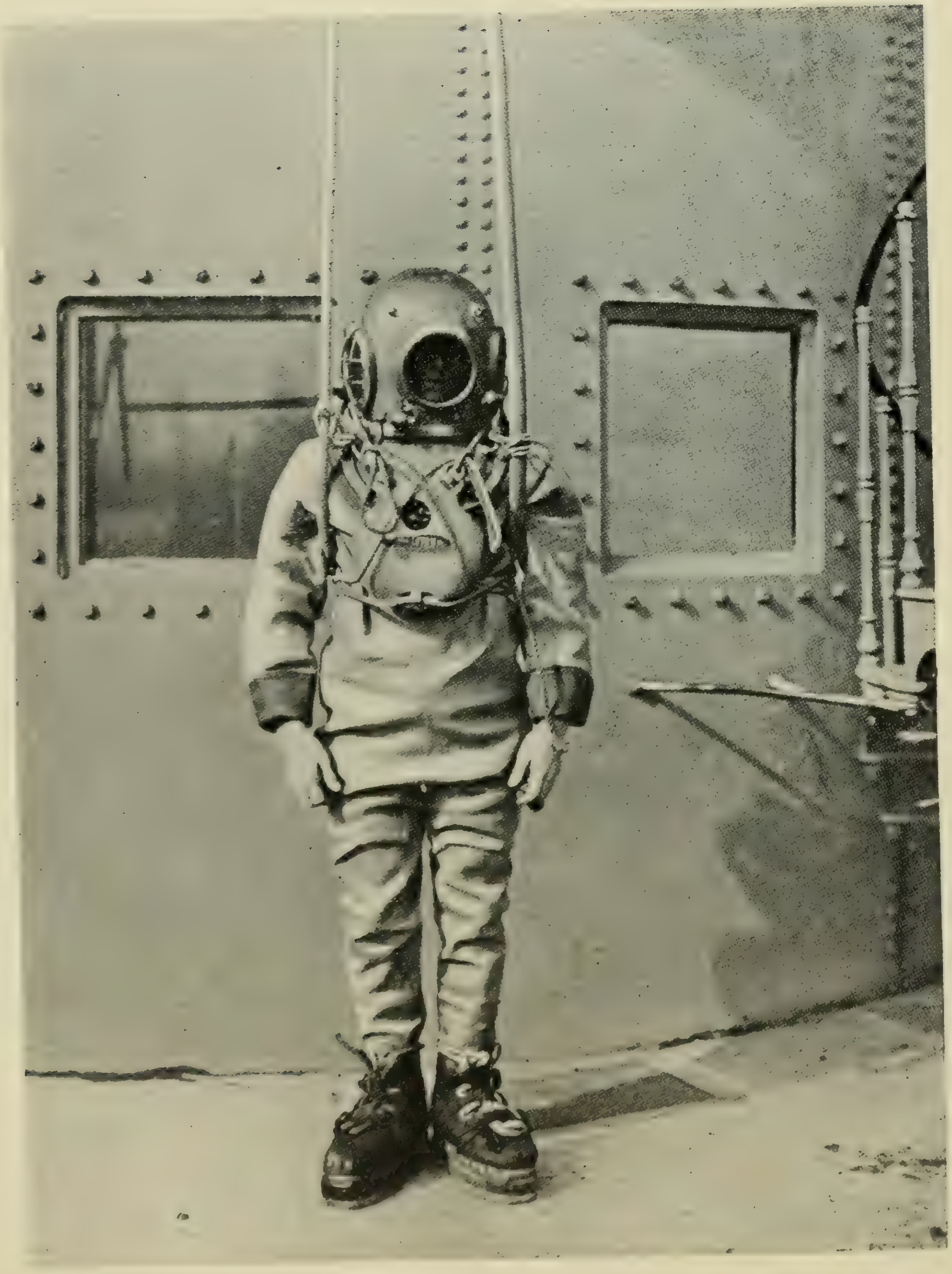
From the foregoing discussion the general nature of the measures needed to prevent compressed-air illness will be evident enough. The risks may best be avoided by properly calculated stage decompression, or by cutting down the period of exposure to a safe limit, or by both methods combined. In the case of work in compressed air in caissons, tunnels, etc., it is for economic reasons very undesirable to greatly reduce the period of exposure. In diving work, on the other hand, the periods of exposure are generally short in any case, and they can, without great inconvenience, be confined within limits which largely reduce the risks of compressed-air illness. Long periods of decompression are also very undesirable in diving, since changes of weather or tide or other causes may render a return to the surface necessary without any long delay in coming up, and since very prolonged stays under water are exhausting, and the diver's hands may become benumbed by cold.

As our investigations were in the first instance made with the object of securing safety from compressed-air illness in diving work, we may first consider the precautions desirable in connection with diving.

(1) *Diving work.*

The ordinary diving dress (Plate IV) consists of a copper helmet screwed to a corselet, the latter being in its turn connected water-tight to a stout water-proof dress covering every part of the body except the hands, which project through elastic cuffs. Air is supplied through a non-return valve on the helmet from a flexible pipe connected with an air-pump on a boat or ship. The air escapes through an adjustable spring valve at the side of the helmet. The arrangement is thus such that the pressure of the helmet air breathed by the diver is always at least equal to, and usually slightly greater than, the pressure of the water at the valve outlet. At a depth of 33 feet or 10 metres the diver is therefore breathing air at an excess pressure of one atmosphere, or at an absolute pressure of two atmospheres; and every additional 33 feet will add another atmosphere to the pressure. To enable the diver to sink, the dress and boots are suitably weighted. He is usually in connection with surface by a life-line containing a telephone wire, as well as by the air-pipe.

PLATE IV



Diving dress, front view, with air-pipe and life-line, which are connected with the helmet behind.

In descending or ascending a diver usually makes use of a rope attached to a heavy sinker at the bottom. He can thus easily regulate the rate of his ascent or descent, and take care that this rate is not so rapid as to cause any discomfort or pain in the ears owing to incomplete opening of the Eustachian tubes. A too rapid descent or ascent might cause mechanical injury followed by middle ear inflammation.

As explained above, there appears to be practically no risk of symptoms occurring from liberation of gas bubbles on rapid decompression if the pressure has not exceeded 1·25 atmospheres, corresponding to a depth of about seven fathoms or 42 feet of sea water. Up to this depth therefore no special precautions against caisson disease need be taken[1]. At greater depths precautions depending on the duration of exposure are evidently needed. The precautions which we have calculated to be desirable are embodied in the table given below (Appendix IV.); and the principles and experimental results on which this table is based must now be discussed.

It will be convenient to consider first the case of diving to a very great depth, and we shall take as an extreme example the case of exposure at a depth of 35½ fathoms (213 feet) of sea water, corresponding to an excess pressure of nearly 6·5 atmospheres, or an absolute pressure of 7·5 atmospheres.

Let us first suppose that the body of a diver is completely saturated with the nitrogen of air at this pressure, and that it is required to conduct his ascent to surface as rapidly as possible but without any risk of symptoms due to bubble formation, *i.e.* in such a way that, in accordance with the principles already laid down, the nitrogen pressure in no part of the body shall ever be more than double that of the air breathed at the same time.

The first step would obviously be to reduce the absolute pressure to about half, *i.e.* from 7·5 atmospheres absolute to 3·75 or from 6·5 atmospheres in excess to 2·75. This would be *ex hypothesi* the greatest initial drop in pressure which would be perfectly safe. The remainder of the decompression would evidently need to be conducted in such a way that the maximum partial pressure of nitrogen in any part of the body should diminish at double the rate of the fall in absolute pressure of the air. The ascent of a diver can be conveniently regulated from the surface by signalling to him to stop or come on at every ten feet as indicated on the pressure gauge attached to the pump. We may therefore divide the ascent into stages of ten feet, and the short periods occupied in the actual ascents may be neglected.

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[1] Heller, Mager and v. Schrötter recommend that at all depths decompression should be at a rate of at least 20 minutes per atmosphere. This would imply a delay of 25 minutes in coming up from 42 feet. Both common practical experience and our own experiments show that this excess of caution is quite unnecessary at small depths.

Since the depth was 213 feet, corresponding to 246 feet of water in absolute pressure, it would be safe to come up at once to a depth corresponding to 123 feet of absolute water pressure, *i.e.* to 90 feet of actual depth. Consequently the first stage would be a rapid ascent of 123 feet, and it would be necessary to wait here before the next ascent of 10 feet until the maximum partial pressure of nitrogen in the body had fallen to that of the nitrogen in air at $2 \times (80 + 33) = 226$ feet of absolute water pressure. The difference between 246 and 226 is 20, and this is 16 °/₀ of $213 - 90 = 123$, the difference between the original and the reduced pressure. The most slowly desaturating parts of the body will, according to our previous calculations, take 75 minutes to give off half of any excess of nitrogen which they may contain at any given air pressure; by inspection of the curve (Fig. 1) it will be seen that they will take about 19 minutes to lose 16 °/₀ of the excess. Hence a delay of 19 minutes would be necessary at 90 feet before coming up to 80 feet. At 80 feet the partial pressure in the body would require to fall an amount corresponding to 20 feet, which is about 17½ °/₀ of $193 - 80 = 113$, the new difference in relative pressure between the nitrogen in the body and in the air. This would necessitate a delay of 21 minutes before ascending to 70 feet. The further delays needed would be 23 minutes at 70 feet, 26 minutes at 60 feet, 30 minutes at 50 feet, 35 minutes at 40 feet, 42 minutes at 30 feet, 51 minutes at 20 feet, and 62 minutes at 10 feet. It would thus take 309 minutes, or more than five hours, to reach surface.

This calculation is represented graphically in Fig. 3. It will be noticed from the figure that the time required for safe decompression does not increase proportionally to the increase in depth. For instance, an increase in depth of 15 feet from 50 to 63 feet necessitates an increase of 45 minutes in the time required for safe decompression; but the same increase in depth from 198 to 213 feet only requires an increase of 15 minutes in the time of decompression.

A somewhat more rapid rate of stage decompression could probably be adopted without appreciable risk to life, but the occurrence under water of even one of the less serious decompression symptoms might be extremely unpleasant or indirectly dangerous, so that a factor which we believe to be thoroughly safe in this respect has been used in the calculation. The possible occurrence of slight symptoms after surface had been reached would not, however, be a serious matter: for this reason half of the last stop at 10 feet from surface might be dispensed with, which would save half an hour. The most slowly desaturating tissues would, according to the calculation, still be only saturated to an excess pressure of 1·3 atmospheres—a safe enough limit perhaps, but leaving no great margin to spare.

Fig. 3 also shows the maximum excess of saturation with uniform decompression in the same time and in 10 hours. It will be seen that uniform decompression in about five hours would leave at the end of decompression an excess saturation within the body of 2·1 atmospheres; and even if uniform decompression were extended to ten hours the excess saturation would still exceed one atmosphere. It is also perfectly clear that uniform decompression is an unsuitable way of bringing a man out of compressed air. Where a sufficiently safe rate of uniform decompression is employed (as, for instance, with 10 hours in the case under consideration), it is only at the very end (when the nitrogen pressure inside the body becomes more than double that of the air) that there is any risk of symptoms occurring; and for the sake of safety at the end the whole process is made quite unnecessarily long. Increased safety at the end is only secured in combination with useless delay at the beginning[1].

As will be seen in Part II, the results of our experiments, allowance being made for the difference between goats and men, fully confirm the foregoing mode of calculation. Not only has stage decompression in the calculated time proved safe where uniform decompression in the same total time was unsafe, but shorter periods of stage decompression than those calculated have been proved to involve risk of symptoms, increasing in gravity and frequency with the shortening of the time, though always less than the risk from uniform decompression in the same time.

If the whole body of a diver were allowed to become saturated at any great depth, it is evident that the time needed for safe decompression would be impracticably long. To reduce the time of decompression to within limits practicable for divers, it is evidently necessary to greatly reduce the period of exposure to high pressure[1]. At great depths limitation of the exposure is also necessary in order to avoid toxic effects from the high pressure of oxygen (see p. 371). Calculation of the mode and period of decompression required after a limited exposure to pressure is a somewhat complicated matter, but the principles already laid down render it quite possible.

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[1] The regulations of the Dutch Government make the following method of decompression obligatory for work in caissons, &c. The pressure is to be lowered at the rate of not more than $\frac{1}{10}$th of an atmosphere in 3 minutes till 3 atmospheres of excess pressure is reached: then at not more than $\frac{1}{10}$th of an atmosphere in 2 minutes till $1\frac{1}{2}$ atmospheres excess pressure is reached; and finally at not more than $\frac{1}{10}$th of an atmosphere in $1\frac{1}{2}$ minutes till normal pressure is reached. This method is still more unsuitable than uniform decompression, and would be very unsafe with high pressures.

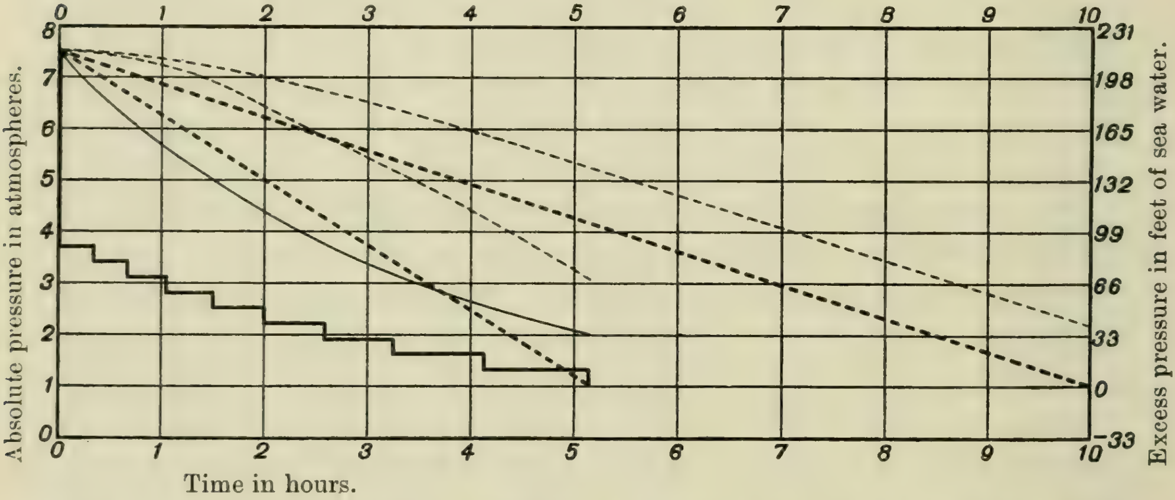

Fig. 3. Theoretical ascents of a diver after a prolonged stay at 213 feet of sea water. Stage decompression in 309 minutes compared with uniform decompressions in 309 minutes and in 10 hours. Continuous lines = stage decompression: interrupted lines = uniform decompression. Thick lines = air pressure: thin lines = saturation with atmospheric nitrogen in parts of the body which half saturate in 75 minutes.

When a diver goes down for a very short time, we have to take into consideration not only the time which he spends at the maximum pressure on the bottom but also the time occupied in the descent and the ascent. During the descent he is all the time saturating himself with nitrogen, and during most of the ascent he may be doing so also. Calculation will show that, if he descends and ascends at a uniform rate, the time spent in this process will be nearly equivalent, as regards the saturation of the body with nitrogen, to half the same time spent at the maximum depth. It is therefore clear that in deep diving the diver should descend as rapidly as is practicable, and should also ascend at once, on completion of his work, as far as he safely can. The rate of descent may be limited either by pain in the ears or by an air supply insufficient to keep the upper part of the dress full of air.

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[1] This was fully realised by Catsaras who recommended a stay on the bottom of only 1 minute at 30 fathoms.

Both these causes are avoidable, and an experienced diver, with his Eustachian tubes well opened and a proper supply of air, can get to an excess pressure of six atmospheres (198 feet) in two minutes. This time was found sufficient in experimental dives up to 210 feet made by Lieut. Damant and Mr Catto (Appendix II). The recommendation commonly made that the rate of both ascent and descent should be slow is evidently quite unsound. A man who spent half an hour in descending to 30 fathoms, and an equal time in ascending at a uniform rate, would run a considerable risk of perishing on his return to the surface.

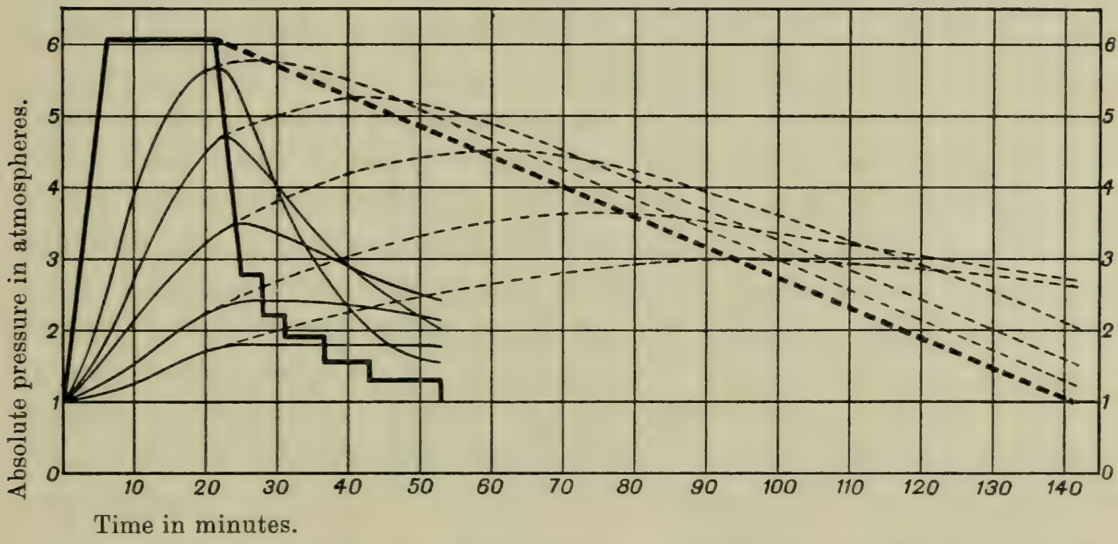

Fig. 4. Desaturation during stage decompression in 32 minutes and uniform decompression in 2 hours, after exposure for 15 minutes at 75 lbs. pressure with compression in 6 minutes. Thick lines = air pressure: continuous lines = stage decompression: dotted lines = uniform decompression. The curves from above downwards represent respectively the variations in saturation with nitrogen of parts of the body which half saturate in 5, 10, 20, 40, and 75 minutes.

In order to illustrate the method by which we have calculated safe modes of ascent in the minimum period of time we may take as an example the case of exposure for 15 minutes to a pressure of 75 pounds (6·1 atmospheres absolute or 28 fathoms = 168 feet). Many of our experiments on goats were made with this pressure and exposure. It took about six minutes to raise the pressure in the experimental chamber to 75 pounds, so that the total virtual exposure till decompression began was about 18 minutes. Fig. 4 shows graphically the variations of pressure during this period: also the calculated partial pressure of nitrogen in different parts of the body, as compared with the nitrogen pressure in the air. The first stage was from 6·1 to 2·8 atmospheres absolute (corresponding to an ascent in sea water from 168 to 60 feet) and occupied four minutes. The subsequent stoppages were:—

2	minutes at	2·8	atmospheres	(60	feet of sea water),
3	„	2·2	„	(40	„	),
5	„	1·9	„	(30	„	),
7	„	1·6	„	(20	„	),
10	„	1·3	„	(10	„	).

It will be seen from the figure that this rate of decompression was slightly faster than what was calculated above to be desirable. At the end of decompression the nitrogen pressure in those parts of the body which became half saturated in about 20 minutes under pressure would be equivalent to that of air at about 1·4 atmospheres, or 20·6 pounds per square inch. If the circulation in one of these parts were less vigorous during decompression than during exposure to the high pressure, it might well be that the nitrogen pressure in this part at the end of decompression would be higher than corresponded to the calculation. As a matter of fact minor symptoms ("bends") were observed five times in 34 decompressions of 18 goats, although no serious effects occurred. We concluded that the period of virtual exposure (18 minutes) was slightly longer than is desirable with stage decompression in 31 minutes: in the table below (p. 442) the limit has been set down at 15 minutes.

Fig. 5 shows the calculated nitrogen pressure in different parts of the body during uniform decompression in 31 minutes after the same exposure at 75 pounds. It will be noticed that at the end of decompression there is a dangerous excess of saturation in all parts of the body except those which half saturate in less than about seven or eight minutes, and that this supersaturation corresponds to an excess pressure of as much as 2·1 atmospheres of air. The goats used for the stage decompression experiments were on alternate occasions subjected to uniform decompression in the same time and with the same exposure. The result was that, in 36 decompressions, one died, two were paralysed, one had indefinite general symptoms of a severe character, and in 11 other cases "bends" occurred, besides two doubtful cases. This was entirely in accord with what the calculation would lead us to expect; and uniform decompression in 31 minutes is evidently dangerous under the conditions given.

It might be supposed that safety would be secured by extending to a moderate degree the length of uniform decompression. It must be remembered however that the more the duration of uniform decompression is extended, the longer is the period during which the body is exposed to high pressure. Fig. 4 shows the calculated effects of uniform decompression extended to two hours. Although the quickly saturating parts of the body are desaturating during the greater part of the decompression, the slowly saturating parts are, on the other hand, becoming more and more saturated, so that at the end of decompression the parts which half saturate in from 40 to 75 minutes are saturated to an excess pressure of about 1·7 atmospheres, although at the beginning of decompression they were only saturated to from 0·7 to 1·3 atmospheres and could consequently have given no trouble.

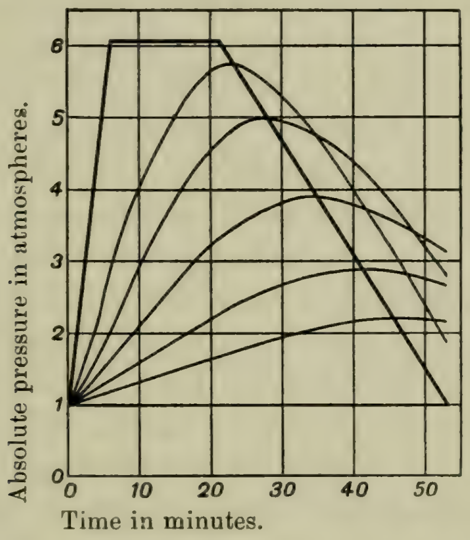

Fig. 5. Desaturation during uniform decompression in 32 minutes after exposure for 15 minutes at 75 lbs. pressure with compression in 6 minutes. Thick line = air pressure. The curves from above downwards represent respectively the variations in saturation with nitrogen of parts of the body which half saturate in 5, 10, 20, 40 and 75 minutes.

Very prolonged uniform decompressions are extremely tedious, and it seemed scarcely worth while to make any extensive series of such experiments. We found however that out of 12 goats uniformly decompressed in 90 minutes after 18 minutes virtual exposure at 75 pounds (6·1 atmospheres of absolute pressure) three developed symptoms of bends after decompression. The proportion of illnesses was thus greater than with stage decompression in a third of the time. With men the results would certainly be much worse, and we calculate that for a man, after the same exposure, several hours would be needed for uniform decompression in order to escape all risk of symptoms occurring. The time would in fact require to be nearly as long as if the body had been completely saturated at the maximum pressure.

With very short exposures to high pressure, rapid decompression is probably safer than uniform decompression at a moderate rate. There is a considerable human experience on this point: divers working at great depths would seem to consider it fairly safe to go rapidly to the bottom at a depth of 160 or 180 feet and return equally rapidly, provided the time spent on the bottom does not exceed six or eight minutes and provided also that the dives are not repeated at short intervals. It is reported of the skilled Greek divers of the Mediterranean that, in case their gear becomes entangled on the bottom, they will cut their air-pipe and line and blow themselves up to the surface in less than a minute from a depth of 30 fathoms or the like rather than stop more than about ten minutes on the bottom. Our experiments on goats are in accordance with this practice. We found that no symptoms were produced by sudden decompression in less than a minute after virtual exposures at 75 pounds up to four minutes, and even in some trials up to six minutes (see below, p. 394).

With exposures exceeding a very few minutes, or such brief exposures frequently repeated, so that during the intervals the body has not time to become desaturated, we have little doubt that slow and uniform decompression—the slower the better—is at any rate preferable to sudden decompression. Uniform decompression must however be extremely slow to make it entirely free from risk of death or very serious symptoms, and the time required is so great that this method seems to us quite impracticable in connection with diving work. There appears to be very little human experience of slow uniform decompression. Divers usually come up in a few minutes at most, and even half an hour spent in the ascent would appear to be quite exceptional. Almost the only definite observations are those of Hill and Greenwood, who recently experimented on themselves at very high pressures. Fig. 6 shows the variations of pressure and the calculated saturations of different parts of the body during the experiment in which Greenwood went to a pressure of 91 pounds (7·1 atmospheres absolute). This experiment appears to have been a very risky one. After decompression he had bends in both arms, and Hill also had symptoms pointing towards blockage of vessels in the subcutaneous fat after similar experiences at 75 pounds pressure.

In Appendix IV two tables are given for the safe decompression of divers after exposure for varying periods of time at different depths. These tables are the same as are now in use for divers in the Royal Navy, on the recommendation of the Committee on Deep Diving. In Table I the period of virtual exposure is so limited that the diver can return to surface by stages in half an hour or less. It will be noted that the maximum periods of exposure are from the time of leaving surface, so that there should be no chance of increased danger from undue delay in descending. The stoppages during the ascent are so calculated that, until surface is nearly reached, the excess of nitrogen pressure in any part of the body should never be more than double the nitrogen pressure of the air breathed, and not more than two and a quarter times this pressure when surface is reached. The only case in which these limits are allowed to be slightly exceeded is with short exposures in comparatively shallow water. This slight excess is, however, only in parts of the body which saturate and desaturate very rapidly, and, as already explained, give rise to no danger. As an additional safeguard the diver is directed to keep his arms and legs constantly moving during each stoppage, so as to increase the rate of circulation and guard against the chance of the rate of desaturation during his ascent being proportionally less than the rate of saturation during his stay on the bottom while he was doing work.

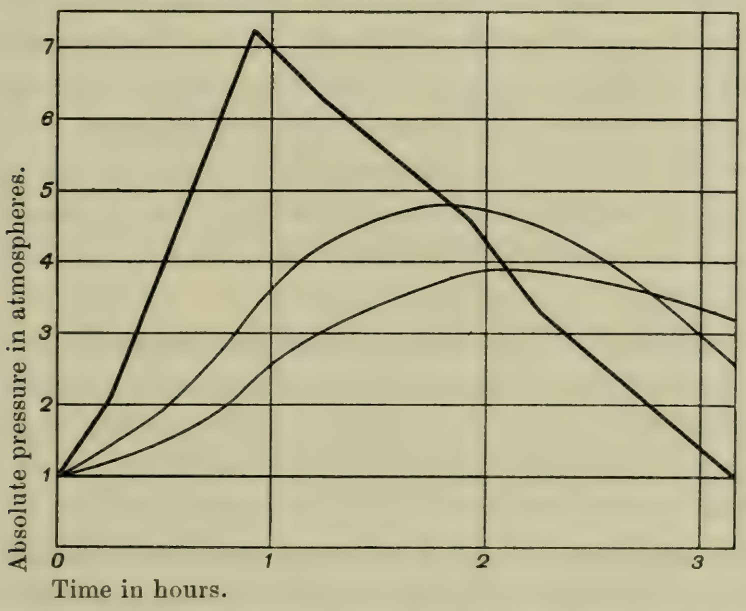

Fig. 6. Showing calculated variations in saturation with nitrogen during Dr Greenwood's experiment on himself. Thick line = air pressure: the two curves from above downwards represent respectively the variations in saturation with nitrogen of parts of the body which half-saturate in 40 and 75 minutes.

The second table provides for the case of exceptionally long stays under water. A diver may be delayed by his air-pipe or life-line being fouled, or by other exceptional circumstances, against which it is necessary to provide. Where the fouling has been complicated by the action of tide the delay on the bottom has occasionally amounted to several hours, until the tide has slackened or turned. If the diver is at a great depth the calculated time required for safe decompression after so prolonged a stay is very long. On the other hand the dangers from cold and exhaustion have to be considered, and the difficulties caused by a strong tide during the diver's ascent. In view of these difficulties the time allowed for decompression after very prolonged exposures is somewhat curtailed, but not so much as to permit of risk of more serious symptoms than "bends," in so far as experiments on animals, and human experience, render it possible to calculate. In the case of men of exceptionally heavy build, and inclined to obesity, the time allowed after very prolonged exposures ought to be increased by about a third, although such men, particularly if over about 45 years of age, ought not to expose themselves to the risk of a prolonged stay in very deep water.

It might appear as if the rate of stage decompression recommended after prolonged exposures was slower than is actually required. A very unfortunate accident which occurred recently has shown only too clearly that this is not the case. In connection with the work of raising a torpedo boat which had sunk in 25 fathoms (150 feet or 46 metres) several divers were employed. They were working in 20 minute spells, and returning to surface by stages in 32 minutes, in accordance with the first table, which was the only one then in use. No symptoms of any kind were observed after the divers' return to surface under these conditions, nor have any symptoms ever been observed hitherto among divers working according to the table. One of the divers, however, became fouled in a very exceptional manner. His life-line was fixed in one direction over a spar or rope belonging to the sunk vessel, and his air-pipe was fixed in the other direction. He was thus prevented from going to free either his air-pipe or life-line. A second diver at once went down, but was unable to free him owing to the drag caused by the tide; and it was only after two and a half hours, when the tide had slackened, that he got free. He was then brought up by stages under the direction of Staff Surgeon Rees and Lieut. Damant. For the decompression two and a half hours were allowed, which we then believed would be a sufficient time in case of a diver being badly fouled at 25 fathoms. The diver was, however, a man of heavy build with much fat in his body, and aged 49. Owing to his exhausted condition he did not come up on the ordinary rope, but had to be pulled up, hanging motionless on the life-line during the long stoppages. On reaching surface he was very exhausted and could hardly have been safely kept longer in the water. He had no paralysis or other definite symptoms of caisson disease. A bed was arranged for him on deck, and hot bottles &c. applied. After a time he complained of some pain in the legs, but this soon subsided; and as he seemed much better in the morning he was removed to hospital, since there was no suitable accommodation for him on the gun-boat where he was. The moving made him worse, and he gradually became restless and delirious in spite of administration of oxygen at intervals, showed signs of cardiac failure, and died somewhat suddenly about 24 hours after he had been brought up. At the post-mortem examination 12 hours later a moderate number of bubbles were found in the right side of the heart, the veins of the liver and intestines, while scattered bubbles were present in vessels elsewhere, including the coronary vessels, though none were seen in the vessels of the brain. The mesenteric fat, which was very abundant, was in places distended with small bubbles. There was about an inch of subcutaneous fat over the trunk, but no bubbles were seen in this layer. There seemed no reason to doubt that death was largely due to the bubbles, although the more usual symptoms of caisson disease were absent. There were no signs of pneumonia.

This is the only known case of prolonged exposure of a man to such a high excess pressure as four and a half atmospheres; and although his age, heavy build, and exhausted condition combined to make the circumstances very unfavourable, the fact of his death shows that the long decompression periods recommended in the second table after prolonged exposures are none too long, even for a man of ordinary build. Every precaution should be taken to guard against such long exposures at high pressures.

A diver has often to descend twice or oftener at short intervals. At the beginning of the second descent the more slowly desaturating parts of the body will not have had time to lose their excess of nitrogen, and consequently they will be more highly saturated at the end of the second descent than would otherwise have been the case. This will be clear from a study of Figs. 2 or 3. To meet the increased risk in decompression it is desirable, in calculating the proper stoppages, to add together the two periods of exposure, and adopt the corresponding rate of decompression shown in the tables. For the first half of the stoppages this is not necessary, but for the second half, including the longer stoppages needed to meet the case of the more slowly desaturating parts, the rule should be carried out. The increasing danger after successive short dives by pearl divers, &c. without any precautions in decompression is notorious. This danger does not mount up to the same extent with stage decompression, but nevertheless exists. As the interval between successive dives increases the added danger on decompression diminishes. With an hour's interval the extra precautions might be halved, and with two or three hours' interval they might be omitted.

It may be remarked that the precautions recommended in the tables are greatly in excess of those which have hitherto been commonly employed in either diving work, or work in caissons, tunnels, &c. We have endeavoured to leave a clear margin beyond everything which either human experience or experiments on animals, or calculation, has shown to be risky. In connection with diving, the practice hitherto recommended in the British and other navies has been that the diver should both descend and ascend at a uniform slow rate. By abolishing the slow descent and ascent, and substituting stage decompression, it has been possible to combine greater safety with a clear saving of time under water for a given working period on the bottom. Where the air supply to the diver is managed in accordance with the recommendations of the Diving Committee there is also very greatly increased working efficiency in deep water. For a discussion of the air supply to divers, and many other practical points relating to diving, we must refer to the Committee's Report; and to the "Diving Manual," which has just been re-written and issued to the Royal Navy.

A possible complication to which we have not hitherto referred in connection with compressed-air illness arises from the fact that at very high pressures of air the partial pressure of oxygen begins to be so high as to be capable of producing serious effects. Paul Bert discovered that oxygen at a partial pressure exceeding about three atmospheres (corresponding to 14·3 atmospheres of air) causes animals to go into convulsions and die, even a short exposure being often fatal. More recently, Lorrain Smith, who experimented on mice, and whose results have been confirmed and extended by Hill and Macleod, showed that oxygen at high pressure acts on the lungs, producing pneumonia[1]. He found that fatal pneumonia may be produced after four days' exposure to an oxygen pressure of as little as 75 °/₀ of an atmosphere, corresponding to air at an absolute pressure of 3·6 atmospheres (88 feet of sea water). At a pressure of about 1·25 atmospheres of oxygen (6 atmospheres of air, or 165 feet of water) death from pneumonia was produced in about 48 hours. At about 1·8 atmospheres of oxygen (eight and a half atmospheres of air, or 250 feet of water), marked symptoms usually occurred in about 12 hours, and death in 20 hours, though in one case death followed in seven hours. At about 2·8 atmospheres of oxygen (13·3 atmospheres of air, or 406 feet of water) marked symptoms were observed in about three hours, and death in nine hours.

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[1] Lorrain Smith, *Journ. of Physiology*, vol. XXIV., p. 19, 1899; Hill and Macleod, *Journ. of Hygiene*, vol. III., p. 401, 1903.

The steel chamber at the Lister Institute was not made to withstand such high pressures as would produce within a short time symptoms of oxygen poisoning if air alone was pumped into the chamber. We have, however, made a few observations in the chamber when the oxygen pressure of the air breathed was raised by other means. In one experiment seven goats were placed in the chamber, and the oxygen pressure raised by opening three large cylinders of oxygen, and at the same time pumping in air to 81 pounds pressure. The total oxygen pressure was thus raised to 2·3 atmospheres, corresponding to a depth of 55 fathoms, or 330 feet, or 100 metres. After three hours one animal had died of pneumonia in the chamber, and most of the others seemed more or less affected, though they rapidly recovered on decompression[1]. We also tried on ourselves the effects of breathing nearly pure oxygen from a bag while we were in the chamber at an absolute pressure of two atmospheres; but we could not detect any effects after a few minutes with an oxygen pressure of 1·7 atmospheres, corresponding to about 40 fathoms (240 feet or 73 metres). In a number of goats which were exposed to 75 pounds' pressure (168 feet or 51 metres of water) for three hours, no symptoms indicative of oxygen poisoning were observed.

To judge from these data there is no immediate risk to a diver from oxygen poisoning at depths up to 40, or perhaps 50 fathoms (73 to 90 metres) if ordinary air is breathed, provided the stay is not long. With stage decompression the diver could rapidly return to a perfectly safe oxygen pressure; but, as already remarked, we do not yet know with certainty whether it is perfectly safe to rapidly reduce the pressure to half after exposure to such very high air pressures.

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[1] One animal showed bends after decompression which was effected in 133 minutes by stages. After exposure at + 75 lbs. for 3 hours in air this decompression gave 2 bends in 14 goats. There is therefore no evidence that the exposure to high pressure oxygen increased the susceptibility to caisson disease.

(2) *Work in caissons, tunnels, and diving bells.*

In connection with various kinds of engineering work under water, or in soft water-bearing strata, compressed air is commonly used for keeping water out of the working place and preventing collapses. The men have thus to work continuously in compressed air.

In tunnels or 'tubes' through soft water-bearing strata, where a steel lining has to be erected to keep water out and resist pressure, the working face, or blind end of the tunnel under construction, is kept free of water by the air pressure with the help of a circular shield with a cutting edge which is advanced as each section of steel lining is erected into position. The soil is excavated by hand labour, and passed out on trucks through an air-lock.

In constructing foundations for the piers of bridges over rivers, caissons are employed. A caisson is a steel tube, which ultimately forms the lining of the pier, and is shaped accordingly. Near the lower end there is a steel diaphragm, forming a working chamber. An inner steel tube passes through this diaphragm, and serves for ingress and egress, and for passing up the material excavated. At the top of this inner tube there are air-locks for allowing the passage of men and material without escape of the compressed air contained in the working chamber. The latter is kept free from water by the air pressure, and the excess of air escapes beneath the cutting edge of the caisson. When a secure foundation for the pier has been reached this chamber is filled up with concrete. In constructing mine shafts through soft water-bearing strata the same principle may be employed. For work of a simpler kind on river or harbour bottoms diving bells are often used, the bell being simply lowered to the bottom at any required place, so that the men can work on the area covered by it and are kept dry by the air pressure.

The circumstances connected with work in compressed air in caissons, tunnels, &c., differ in certain respects from those associated with diving work.

In the first place the duration of exposure is far longer. A caisson or tunnel worker is usually in compressed air for six or eight hours daily, or even longer. The conditions of the work render any great limitation of the periods of exposure very difficult and expensive.

Usually, however, the workman comes out for meals at intervals of about three hours.

A second difference is that the very high pressures to which a diver may have to go are not needed in caisson or tunnel work. An excess pressure of about 3¼ atmospheres, or 48 lbs., is, we believe, the extreme limit hitherto employed; and usually the excess pressure does not exceed about two atmospheres or 30 lbs. Decompression seems to be usually effected in 10 to 20 minutes, or even, with the lower pressures, in three to five minutes.

With properly arranged air-locks for men and material there should be no need for hurry in coming out; and undue hurry is specially undesirable if the workman leaves the works at once, since he would be liable to develop symptoms when he was so far away that he could not be readily recompressed. To obviate this risk as far as possible, it is customary to endeavour to keep men for half to one hour on the works after they come out; and with the usual rates of uniform decompression this precaution is very necessary. Evidently, however, it is greatly preferable to prevent all practical risks of serious symptoms.

In order to attain this end stage decompression as recommended for divers in the tables in Appendix IV may be employed. An accurate and easily read pressure gauge, visible from both inside and outside the air-lock, is of course essential; and a reliable man should be in charge of the tap. As a further control it would be desirable to have an automatic graphic record of the variations of pressure each time the lock for men is used. As any very sudden drop in pressure might cause mechanical injury, the outlet tap should be so arranged as to prevent decompression at a maximum initial rate of more than about one pound in five seconds[1]. With this arrangement and an ordinary tap, the rate of decompression would diminish considerably as the pressure fell, and the proper point for interrupting the decompression could be accurately reached.

The tables in Appendix IV have been calculated with special regard to the comparatively short periods of exposure to pressure in diving work;

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[1] The delivery of the inlet tap should also be restricted, and the man in charge should have strict directions to take care that the rate of admission or discharge of air does not cause pain in the ears, &c. of any of the men in the lock. To avoid pain a very slow rate of air admission may sometimes be needed, but with practice a rise of pressure of one atmosphere per minute is often not too much, so that any definite rule, limiting the rate to much less than this, seems scarcely desirable.

and the stoppages recommended during the divers' ascent after exceptionally long periods of exposure are somewhat shorter than would be desirable apart from the risks entailed by the long stay under water. In the case of caisson and tunnel workers, on the other hand, it is only in exceptional cases that the exposure to pressure lasts less than three hours; and usually the exposure during the day lasts at least six hours.

With such long exposures and only moderate pressures the calculated theoretical rate of safe decompression after the first rapid stage is nearly uniform; and the rules for decompression may be greatly simplified by adopting uniform slow decompression or uniform stages[1].

The following table shows the rate of uniform slow decompression calculated to be safe after the initial diminution of absolute pressure in the proportion of 2 : 1. Suppose, for instance, that men were working at a pressure of 24 pounds in 3-hour spells, with an hour's interval between for a meal. In coming out they would be rapidly decompressed to an absolute pressure of $\frac{24+15}{2} = 19{\cdot}5$ pounds or 4·5 pounds of excess pressure. After the first 3-hour spell of work the slow decompression would be at the rate of one pound in three minutes, or $3 \times 4{\cdot}5 = 13\frac{1}{2}$ minutes in all. After the second spell the rate would be one pound in five minutes, corresponding to $22\frac{1}{2}$ minutes in all. If they stayed for the whole period in the compressed air the rate of slow decompression would be one pound in seven minutes corresponding to $31\frac{1}{2}$ minutes in all. To take another example, if the work were at 40 pounds excess pressure the men could be rapidly decompressed to $\frac{40+15}{2} = 27\frac{1}{2}$ pounds of absolute pressure, or $12\frac{1}{2}$ pounds excess pressure. After a first 3-hour spell of work the period of slow decompression would therefore be $12\frac{1}{2} \times 7 = 87$ minutes: after a second spell (with an interval of 30 or 45 minutes outside the lock) $12\frac{1}{2} \times 8 = 100$ minutes; and after a continuous exposure of six or seven hours, $12\frac{1}{2} \times 9 = 112$ minutes[2].

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[1] With the lock air-tight, and no ventilation, uniform decompression at any required rate could be easily secured by means of a reducing valve on an outlet, with a graduated tap beyond it, the arrangement being similar to the reducing valve and tap usually connected to a cylinder of compressed oxygen or gas used for limelight. If the delay in the lock is so long that ventilation is required, or if ventilation is needed in order to compensate for accidental leakage, it would be best to have an adjustable safety valve on the outlet, and adjust this by one pound at a time at the proper intervals.

[2] We have some doubt as to whether the increased slowness of decompression after very long exposures would be altogether sufficient to meet the increased tendency to slight symptoms ("bends"). These are, however, of minor importance if all serious symptoms

TABLE I.

Table showing rate of decompression in caisson and tunnel work.

Working pressure in pounds per square inch	Number of minutes for each pound of decompression after the first rapid stage		
	After first three hours' exposure	After second or third three hours' exposure, following an interval for a meal	After six hours or more of continuous exposure
18—20 pounds	2	3	5
21—24 ,,	3	5	7
25—29 ,,	5	7	8
30—34 ,,	6	7	9
35—39 ,,	7	8	9
40—45 ,,	7	8	9

It will be evident from the last example that in order to avoid waste of time in the lock it would be preferable with pressures exceeding about 25 pounds to keep the men under pressure continuously during each shift. Thus with two 3-hour spells of work separated by a decompression, the time spent in the lock would be $87 + 100 = 187$ minutes; whereas if the meal were taken in the compressed air, the two 3-hour spells would only imply 112 minutes in the lock.

With working pressures exceeding about 25 pounds the air-lock should be roomy and comfortably arranged, and large enough to take the whole of a shift of men. It should be provided with an electric heater, telephone, and if possible some sort of lavatory accommodation.

With pressures up to 45 pounds, or four atmospheres of absolute pressure, there appears to be no substantial objection to keeping men for six hours, or even more, continuously under pressure, provided that the mode of decompression is thoroughly safe. With pressures exceeding about 40 pounds, the practice has hitherto been to limit the exposure to about one hour, and employ rates of decompression which are dangerously rapid. This plan implies greatly increased risk and expense, since for the accomplishment of the work the number of decompressions is six times as great, and the men are idle most of the day. The actual increase in risk must be very great.

In tunnel work, or any other kind of work where plenty of space is available, there would be great advantage in providing a large air-lock, or section of tunnel, in which the pressure was constantly maintained at a little less than half the absolute pressure in the working section.

are prevented. We also think that with long shifts, exceeding a total of about 3 hours, still slower decompression would be needed for any men inclined to obesity. Such men should, therefore, be excluded in the medical examination which all men working in air at high pressures ought previously to undergo.

The men could then pass rapidly (in two or three minutes) from the working section into this intermediate lock or section, where they could take their meals, wash, and change their clothes. After a sufficient delay (dependent on the working pressure) they could then pass out rapidly. If, for instance, the working section was at a pressure of 30 pounds, the intermediate or "purgatory" lock could be kept at an absolute pressure of about $\frac{30 + 15}{2 \cdot 2} = 20 \cdot 5$ lbs., or $5\frac{1}{2}$ pounds of excess pressure[1]. At the end of the day's work there would be a delay of about 50 minutes in this large lock, during which the men could wash and change, or take a meal. With this plan all delays during actual decompression would be obviated, so that ingress and egress would be free at all times, and the men could use the locks employed for material. For persons going in for only short periods the delay in the "purgatory" lock could be curtailed in accordance with the tables in Appendix IV. The movement of the men while employed in washing, changing clothes, &c. would hasten the process of desaturation, and this would be a further advantage.

In any case where it was specially desirable to reduce the period of delay in the air-lock to a minimum, recourse could of course be had to breathing oxygen during the period of slow decompression. This would about double the rate of desaturation, and therefore halve the delay. The oxygen could be breathed from a bag, and the CO_2 absorbed by a purifier, so that very little oxygen would be needed. By so arranging the mouthpiece that part of the expired CO_2 was rebreathed, and the respiration and circulation thus stimulated, a still better result would be attained.

The results of some of our experiments seem to indicate that even the very slow rate of stage decompression which has been recommended above would be insufficient to completely obviate the risk of "bends" occurring after prolonged exposure. The rate of saturation and desaturation of some of the tissues which are the seat of "bends" is possibly slower than we have provisionally assumed. What we have aimed at is to completely obviate the risk of any serious symptom, while at the same time reducing the chances of "bends" to a minimum.

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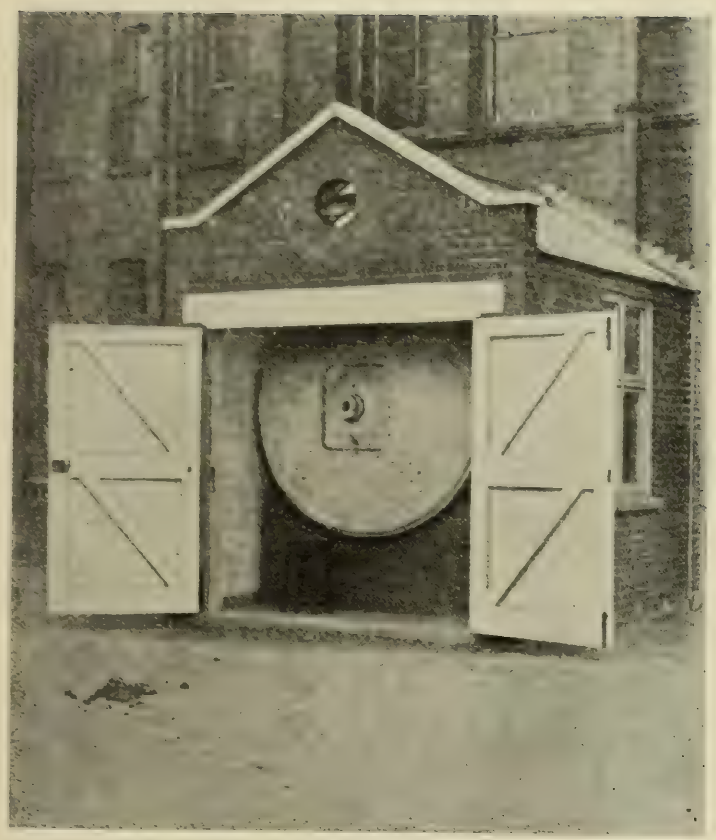
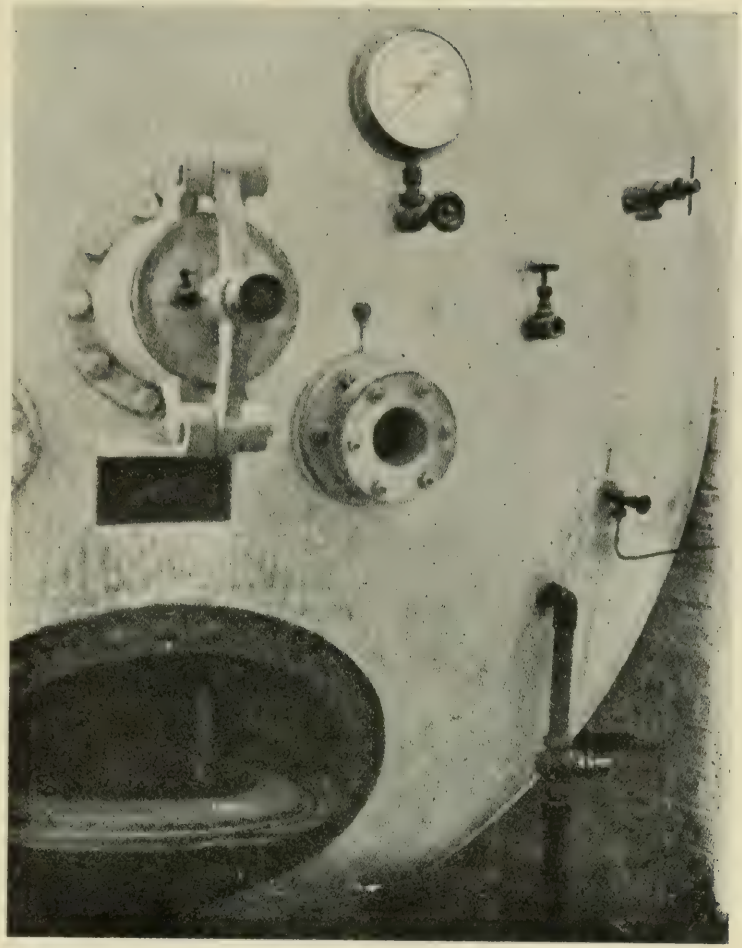
[1] A comparatively rapid fall in absolute pressure in the proportion of 2·2 to 1 is within practically safe limits, particularly if the previous period of continued exposure has not exceeded three or four hours.



The steel chamber at the Lister Institute. View from outside, showing the back end of the chamber, with the large door and one inspection window.



The steel chamber at the Lister Institute. Front end, showing the manhole for entering, the small air-lock for passing food, &c. into the chamber, an inspection window, a pressure gauge, and several valves, &c.

PART II. EXPERIMENTAL.

1. *Apparatus.*

We owe the large pressure chamber (Plate V) in which both human and animal experiments were conducted to the generosity of Dr Ludwig Mond, F.R.S. It is a short segment of a boiler of $\frac{5}{8}$ inch plate resting on its side; the ends are slightly dished steel plates $\frac{1}{2}$ inch thick. Inside it measures $7\frac{1}{2}$ feet long by 7 feet wide and high, and has a capacity of 9500 litres (336 cubic feet). It is thus large enough to hold 3 or 4 persons comfortable and can be used for animal experiments lasting several hours without the necessity of ventilating. There are two doors: one, an oval manhole (24 × 15 inches), is easily removed and is in common use; at the other end is a large rectangular plate (28 × 24 inches) which can be unbolted for the admission of bulky articles. There are a number of spring and simple valves; the largest is in the floor of the chamber and serves also as a drain; when fully opened it reduces the pressure from 100 lbs. to atmospheric pressure in rather less than a minute. Besides this there are four spring and three simple valves so arranged that the pressure can be completely controlled either from inside or outside. The front is also furnished with an air-lock, by means of which small articles can be passed in or out of the chamber during an experiment. Three windows are provided of stout glass; as a precaution for safety these are fitted with an arrangement whereby the breaking of the glass releases a solid metal rubber-faced plug which falls into the hole. Wiring for lights, a telephone, electric heaters and a motor to drive a fan, kymograph, &c., is introduced through fibre plugs.

The pressure is raised or reduced by a simple compressor driven by a gas engine. While this has proved quite satisfactory for negative pressure experiments, the rate at which the pressure can be raised by its means is only about 2 lbs. per minute. This was a serious obstacle to the examination of the effects of exposure to high pressures of short duration. Accordingly after the preliminary experiments, a multitubular compressed air reservoir was placed at our disposal by the Admiralty. This reservoir has a capacity of about 22 cubic feet, and by charging it to about 70 atmospheres with a two-stage liquid-air compressor and also another steel bottle to 180 atmospheres we were enabled to suddenly blow the contents into the chamber and so reach a pressure of 60 lbs. in

4 minutes, and 75 lbs. in 5½—6½ minutes according to the temperature. The pressure is indicated by two Schaffer spring gauges (one of which is visible from within the chamber) for positive pressures, and one spring gauge outside and a mercurial barometer inside for negative pressures. The spring gauges show a lag of nearly 2 lbs. up to about half an atmosphere, but above one atmosphere they are concordant and, as far as could be ascertained, correct.

The chamber and accessory apparatus have now been frequently used during eighteen months for experiments at pressures varying from 100 lbs. above to 8 lbs. below atmospheric pressure, and have been found very satisfactory and convenient.

2. *Choice of experimental animals.*

A few experiments were made with rabbits, guinea-pigs, rats, and mice, but for regular use goats were selected chiefly because they were the largest animals which could be conveniently dealt with and which could be obtained in considerable numbers. The questions under consideration depend in a very fundamental way upon the rate of circulation in the animal under investigation. Among the ordinary mammals this must vary with the rate of the respiratory exchange per unit of body weight and is therefore proportional to the ratio between body surface and body weight. The susceptibility of any animal to caisson disease after sufficiently long exposure to compressed air must depend in the main upon the rate at which its respiration and circulation removes the excess of dissolved nitrogen on decompression.

Not only is this excess removed more rapidly in small animals, so that the time during which bubbles might be formed is correspondingly less, but, as already pointed out, there is every reason to believe that the time during which the venous blood remains in a supersaturated state during each round of the circulation largely determines the formation of bubbles. This time is so short in small animals that no bubbles at all are formed, in spite of the temporary existence of very great supersaturation in the blood and tissues. The susceptibility of any species of animal then varies enormously with the size. Thus a mouse, weighing 20 grammes and with a CO_2 production of about 8 grammes per kilo per hour, is much less susceptible than a goat, weighing 20,000 grammes and producing about 0·8 gramme CO_2. We have indeed failed to produce any symptoms at all in mice on decompression in less than a minute after one hour's exposure at 75 lbs., an experience invariably fatal to goats. In the same way dogs, with a respiratory exchange of some 1·3 gms. CO_2 per kilo per hour, are much less susceptible than men with an exchange of about 0·5 gms. Thus Heller, Mager and v. Schrötter observed no symptoms in dogs[1] on sudden decompression from any pressure less than about 60 lbs., while abundant illnesses are caused in man, and for the matter of that in goats also, by inappropriate release from 30 lbs. pressure. It therefore appears clear that it is necessary to use large animals for experiments which are designed to illustrate the incidence of caisson disease in man. Indeed the quantitative factor by which the results obtained on quite small animals might be translated into human experience is so large as to become qualitative in character.

Since pressures of some 100 lbs. or more are required to produce symptoms in a reasonable proportion of small animals, the use of animals such as goats is also very desirable in order to keep as far away as possible from the point at which the partial pressure of oxygen is high enough to cause toxic effects. We found that an exposure of three hours at 81 lbs. to an atmosphere containing 36 °/₀ oxygen (the oxygen pressure being thus equal to that of 150 lbs. excess air pressure) killed one goat out of seven with "pneumonia." Our experience shows that it is not necessary to exceed an air pressure of half this (75 lbs.) to produce symptoms which are sufficiently varied and severe to satisfy experimental requirements.

Experience also showed that goats were very suitable animals in that slight symptoms were presented to our notice in a definite objective form. The lesser symptoms of caisson disease cannot be neglected, and there are reasons for supposing that their occurrence is not exactly conditioned by those experimental circumstances which in a more severe form produce serious and fatal results. They cannot be properly detected in mice or guinea-pigs or even in rabbits. Goats, while they are not perhaps such delicate indicators as monkeys or dogs, and though they are somewhat stupid and definitely insensitive to pain, are capable of entering into emotional relationships with their surroundings, animate and inanimate, of a kind sufficiently nice to enable those who are familiar with them to detect slight abnormalities with a fair degree of certainty.

The animals, 85 in number, used in the present experiments were a mixed collection of ordinary English goats of no particular breed. They were about equally distributed between the sexes, and varied in weight from 10 to 30 kilos, the average being rather less than 20 kilogrammes. All were apparently adult, judging from the fact that none showed any increase in weight while in our possession. One or two (XIII A, XXXII A) seemed to be quite aged, but the rest were fully active.

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[1] The weights are only given in a few instances; from these it may be surmised that the dogs were small (5 to 12 kilos).

On the whole the herd remained healthy. Two died of apical pneumonia and two of diarrhoea, which was at one time epidemic in a severe form. The cause could not be determined, but the trouble became much less marked after the animals were placed on a more meagre diet and corn withheld. Three animals were under some suspicion of being infected with *M. melitensis*[1]; two of them seemed rather depressed (though not more so than appears to be natural in some goats), while the third showed no signs of ill-health. Various items of pathological interest were found in those which came to post-mortem: in the lungs various nematodes were found several times, *Linguatula* once, and a surgical needle once; a *Streptothrix* abscess in the stomach wall followed puncture with a needle to relieve distension; a bony tumour was found in an adrenal gland; in one old goat (XXXII A) the aorta was extensively atheromatous; flukes occurred in the liver once, while hydatids in the peritoneum were very common and intestinal worms abundant. None of these conditions (except possibly the arterial disease) can however be considered to have rendered the animals definitely abnormal as far as caisson disease was concerned, and none of them could be attributed to exposure to compressed air.

3. *Respiratory exchange of goats.*

The difficulties of measuring directly the circulatory activity of normal animals are almost insuperable. This must be however in general proportionate to the rate of respiratory exchange, and a number of determinations of the CO_2 production of our goats were made in order to get a line of comparison with other animals (and especially man) in respect of the rate at which air would be taken up by and discharged from the body.

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[1] Of 22 animals whose blood was examined, 16 gave no reaction with *M. melitensis* at a dilution of 1 : 20, 3 gave some reaction at 1 : 20, while 3 animals gave complete agglutination up to 1 : 200 (XVIII A, XXVI A, XXIII A). Cultures from the blood during life were negative, and when they eventually came to autopsy cultures of blood, spleen, liver, inguinal, axillary, mesenteric and mammary glands were negative as regards *M. melitensis*. The exact history of these animals could not be obtained, but there is practically no doubt that they had never been out of England.

The observations were made by using the pressure-box as a respiration chamber. The animals were enclosed and hourly samples removed (after thorough mixing with an electric fan) and analysed in a delicate form of Haldane's gas analysis apparatus. The results were entirely satisfactory, the successive analyses showing a regular increase in the CO_2. The goats led a regular life, and all the observations were made at approximately the same time of day so that they are fairly comparable with one another in respect of the influence of food. The animals remained fairly quiet, though they seldom lay down.

The results of 27 experiments are given in the next table. The analyses have been calculated in grammes of CO_2 per hour per kilo of

TABLE II.

					Goats					CO_2 gms. per hour		
Number of experiment	Temp. °C.	Bar. mm.	Duration hours	Pressure lbs. positive	Number	Males	Females	Total weight kilos.	Average wt. kilos.	Per kilo body wt.	Per 1000 sq. cms. surface	Remarks
I	19	764	3	0	4	—	4	99·0	24·7	1·006	2·625	
II	13	763	2½	0	4	2	2	62·8	15·7	1·123	2·070	
III	15·5	765	1½	0	9	4	5	159·7	17·7	0·908	1·823	
IV	15	752	1½	0	9	3	6	171·1	19·0	0·727	1·749	
V	17	762	3	0	6	6	—	111·8	18·6	1·104	2·554	R. Q. 1·03.
VI	11	769	6	0	6	—	6	138·8	23·1	0·670	1·771	R. Q. 0·90.
VII	13	765	5	0	6	6	—	121·3	20·2	0·975	2·389	R. Q. 1·06.
VIII	13	754	4	45	7	7	—	142·7	20·4	0·887	2·187	
IX	13	740	4	45	8	—	8	193·7	24·2	0·627	1·682	
X	15	754	7	0	7	7	—	142·1	20·3	0·664	1·630	R. Q. 0·91.
XI	16	780	6	0	6	6	—	126·3	21·0	0·615	1·533	Fasting 20 hrs.: R. Q. 0·82.
XII	15	778	4	21	7	7	—	140·9	20·1	0·763	1·770	
XIII	15	774	7	0	8	—	8	193·7	24·2	0·548	1·469	R. Q. 0·82.
XIV	14	760	4	45	13	5	8	295·7	22·7	0·667	1·738	R. Q. 1·08.
XV	16	758	3½	25	6	6	—	127·9	21·3	0·959	2·367	
XVI	17	764	3⅔	20	7	3	4	148·7	21·2	0·635	1·572	
XVII	15	762	4	0	6	6	—	127·9	21·3	0·669	1·652	
XVIII	17	762	1¾	45	6	—	6	122·1	20·3	1·020	2·504	
XIX	14	761	4	45	6	6	—	127·9	21·3	0·697	1·722	
XX	13	760	3	0	5	—	5	100·1	20·0	0·921	2·258	
XXI	16	760	4	45	5	—	5	100·1	20·0	1·104	2·701	
XXII	15	762	4	45	6	6	—	127·9	21·3	0·967	2·390	
XXIII	16	768	4	45	5	—	5	100·1	20·0	0·852	2·083	
XXIV	12	776	5	0	4	2	2	95·3	23·8	0·717	1·853	
XXV	14	775	5	0	4	2	2	95·3	23·8	0·751	1·941	
XXVI	14	775	5	0	4	1	3	76·9	19·2	0·624	1·501	
XXVII	13	766	5	0	4	1	3	76·9	19·2	0·704	1·693	

body weight and also per 1000 square centimetres of surface according to the usual formula $S \times 100 = \sqrt[3]{W^2} \times 11{\cdot}2$, where S = surface in square centimetres and W the body weight in kilogrammes.

The goats used belonged to Series II (Exps. 1—4), III and IV.

The results of these experiments are very variable; the averages are shown in the next table:

TABLE III.

		CO_2 in grms. per hour	
	No. of experiments	Per kilo body-weight	Per 1000 sq. cms. surface
At atmospheric pressure	16	0·795	1·907
At 45 lbs. positive	8	0·853	2·126
At 20, 21 and 25 lbs. positive	3	0·786	1·903
All pressure experiments	11	0·834	2·065
Males only	10	0·830	2·019
Females only	8	0·843	2·137
Mixed experiments	9	0·762	1·771
All experiments	27	0·811	1·971
		(410 c.c.)	(997 c.c.)

One may conclude that goats produce about 0·8 grm. CO_2 per kilo per hour under conditions of incomplete rest, and that no great departure from this figure is occasioned by the animals being under pressure up to 45 lbs. or by sex. It is shown elsewhere[1] that something more than 10 % of the total CO_2 produced by goats comes from the fermentation of the contents of the alimentary canal, and figures detailed below (p. 409) indicate that one-fifth of the body weight is contributed by these contents. In comparing the CO_2 production of goats with that of man, we may regard these two corrections as roughly balancing one another and may neglect them.

It appears that man produces under conditions of bodily activity comparable to that of our experimental animals, about 0·45 to 0·5 grm. CO_2 per kilo per hour. Goats therefore show a respiratory activity approximately 1·7 times that of man. This figure corresponds fairly well with that calculated from the size. If the respiratory exchange per unit of surface is the same, a goat of 20 kilos will produce 1·5 times as much CO_2 per unit of weight as a man of 70 kilos.

4. *Method of conducting the experiments.*

No animals were subjected to experiment when obviously ill. As a rule five to eight animals were put in at one time. The pressure having been raised to the desired point, the chamber was entirely closed and no ventilation given until decompression began. The average CO_2 production of goats is about 435 c.c. per kilo per hour at ordinary temperatures. The chamber usually contained 100 to 150 kilos of goat so that the CO_2 rose about 0·45 to 0·55 % (measured at atmospheric pressure) per hour. In this way it never attained a harmful partial pressure in experiments lasting from a few minutes to four hours. In the few observations made with an exposure of eight hours, the CO_2 was allowed to accumulate for four hours and afterwards the chamber was ventilated so that the CO_2 did not exceed a partial pressure of 2 % of an atmosphere. No experiments have been made to directly examine the possible influence of CO_2 upon the incidence of caisson disease. It appears to the authors that the effect must (1) in any case be very slight with partial pressures of less than 2 or 3 %, and the result, if any, of the increased respiratory and circulatory activity must be in the direction of diminishing the ill-effects of decompression after any but quite short exposures[1].

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[1] *Journal of Physiology*, vol. XXXVI. (1907), p. 283.

After the preliminary experiments (Series I), the animals were never used more than once on the same day, and, with rare exceptions, not on succeeding days. In many cases indeed individual goats rested for a week or more between the experiments.

During decompression the animals could be watched fairly satisfactorily through the windows of the chamber, though fog of course completely blocked the view during the actual moments of rapid decompression. At the end they were allowed to escape from the chamber and run about free in the yard. They were kept under continuous observation for half an hour or longer, and were frequently seen throughout the day. We found that practically all the symptoms which were going to appear declared themselves within thirty minutes, though a few slight signs were probably missed. We also found that slight signs were much more obvious when the animals were not distracted or excited by food or other causes. During the breeding season it is advisable to keep the males and females separate, and, by removing any sources of interest, to allow the animals to fall into a state of meditative boredom. Under these circumstances, trivial symptoms are easily detected which are not made the subject of objective demonstration by animals engaged with their appetites.

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[1] Greenwood (*British Medical Journal*, June 22nd, 1907, Supplement, p. 409) has recently found that high percentages of CO_2 do not increase the liability to decompression symptoms.

No observations were made of the temperature within the chamber during an experiment. Very hot and very cold weather did not seem to influence the results. The air in the chamber was always warmed by compression and sometimes also artificially, while decompression was of course accompanied by sudden, often very severe, spells of cold. No account has been taken of variations in atmospheric pressure. The extreme readings of the barometer on record are 806 and 689 mm. at sea level, and in this country 790 and 695 mm.[1], giving ranges of 117 mm. and 95 mm. or about $2\frac{1}{4}$ and $1\frac{4}{5}$ pounds. Even this variation, though it occurs at an important part of the absolute pressure scale, cannot be of great significance.

Times of exposure of one hour or less are, unless the contrary is directly specified, to be taken as indicating actual exposure to the given pressure, the time of compression (six minutes) being neglected. For longer exposures it was sometimes convenient to raise the pressure more slowly: in these cases therefore the times specified may indicate either the actual exposure *plus* four to six minutes compression or a virtual exposure calculated by adding the actual exposure to half the time of compression which is in minutes roughly one quarter of the pressure in pounds positive (see above, p. 362).

As will be gathered from the details given below, the general scheme of the experiments involved the examination of three variable factors—degree of pressure, duration of exposure and duration and mode of decompression. For the most part the degree of pressure was kept constant while the other two factors were varied. It soon appeared from the preliminary experiments that the individual variability of the animals was very large—larger indeed than the difference between many of the modes of decompression which it was desired to examine. It also appeared that the relative susceptibility of the different individual animals remained fairly constant so that after a time one could pick out goats which were known to be either susceptible above the average or definitely resistant to caisson disease. It was therefore clear that either an enormous number of animals had to be employed or the experiments had to be so framed as not to produce fatal results and so reduce the proportion of susceptible individuals in the herd. It appears probable that any 20 or 30 goats would give much the same results, but if many are lost it is necessary to discard the remainder and procure a fresh batch to be subjected to the comparative experience. Obvious reasons prevented this procedure. It was therefore necessary to be at

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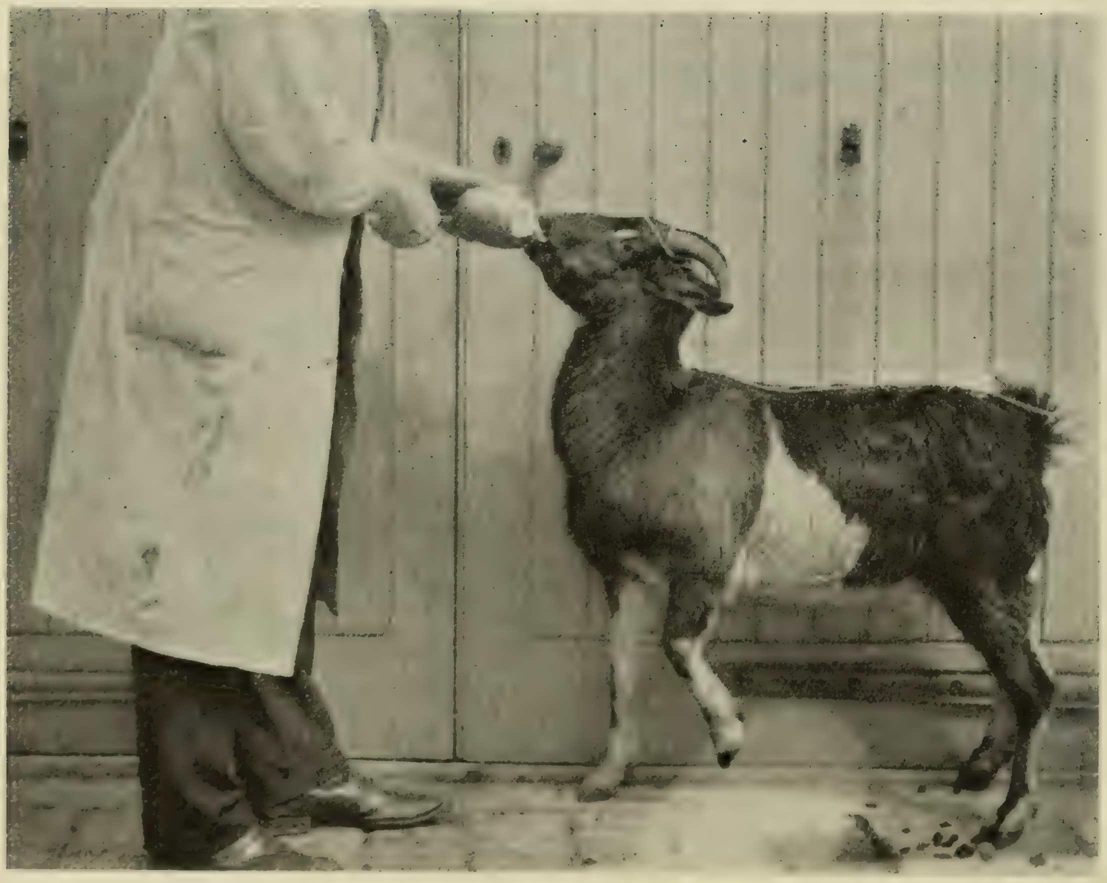
[1] *Nature*, vol. LXXV., 1907, p. 330.



"Bends" of fore-leg in a goat.

some pains to secure that the deaths should be as few as possible so that the same individuals might pass through a number of different combinations of pressure, exposure and decompression. The animals were therefore first put through the experiments which we surmised would give least symptoms, and were subsequently exposed to circumstances of progressively increasing severity. Even so, each batch of animals became selected to a more or less considerable degree. Be it noted however that one may in this way obtain strong evidence of an *a fortiori* kind. For, if the selected resistant members of the herd show many symptoms in the severest experiments, so much the more would the whole original lot of average animals have been affected. This individual variability of the animals renders many of our experiments incomplete, and should be constantly borne in mind in considering the results obtained.

5. *The symptoms observed in goats.*

The symptoms observed in goats in sequence to decompression are protean in character. The majority may however be grouped under a few definite heads.

1. *Bends.* The commonest symptom which we have observed consists of the exhibition of signs indicating that the animal feels uneasy in one or more of its legs. The limb, most commonly a fore-leg, is held up prominently in the air and the animal is evidently loth to bear weight upon it (see Plate VI[1]). In mild cases such a limb is used normally in walking or running, but in other instances the animal limps more or less considerably when it is forced to use the affected member, and is often very anxious to lie down. No tenderness can be detected on pressure or manipulation of the leg and it is not altogether clear that the animal suffers definite pain. We have however noted that a goat may break its leg and immediately use it for progression without evincing any signs of pain. We may conclude from this that the response to stimuli which in many animals would be distinctly painful is largely suppressed in the goat to the level of the exhibition of a consciousness that the limb is somewhat abnormal and not well suited for active use. But it must be understood that this objective demonstration is a very conspicuous and definite symptom. There is little doubt that these symptoms observed in the legs of goats are the equivalent of the "bends" or "screws" which are the commonest

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[1] We are indebted to Dr H. W. Armit for this photograph.

symptoms in caisson workers; in human experience they are of course accompanied by definite pain, often of a severe character.

The following table shows the distribution of "bends" in the last 110 cases observed:

TABLE IV.

One hind leg ...	28
One fore leg ...	70
Both hind legs...	1
Both fore legs ...	1
One fore and one hind leg ...	10
Total one leg ...	98
Total two legs ...	12
Total right ...	50
Total left ...	48

"Bends" may be seen immediately at, or indeed (but very rarely) shortly before the end of a long decompression. Most commonly however they come on after an interval of about 15 minutes; on the other hand they may be delayed still more. As might be expected, the period of delay varies with the duration of decompression: thus the average delay in a number of cases after rapid decompression (1 to 10 minutes) was 16 minutes, which was reduced by long decompression to six minutes. Their duration appears to be brief; all evidence of their presence has usually disappeared in one or two hours and it has been very exceptional for any trace of them to be present next day (16 to 20 hours).

"Bends" in parts of the body other than the limbs are very difficult to identify in animals; we have however occasionally noted symptoms which might well be bends in the trunk, though we are not prepared to definitely identify them as such.

2. *Temporary paralyses* may be of two kinds. In the first a general weakness is present accompanied with dyspnoea and there is dragging of the hind legs with foot-drop. These are clearly symptoms due to a general deficiency of oxygen from pulmonary embolism and are comparable to the paralyses seen in, *e.g.*, carbon monoxide poisoning in animals and men. In our records and the tables such cases are not classified as "paralysis" but as "dyspnoea." In the second group fall a series of cases which are obviously of nervous origin. The animal, while showing no signs of general illness, or in other instances having already had bends, exhibits foot-drop or a more extensive palsy in one or more hind- or fore-limbs. The paralysis does not usually come on till about 15 minutes after decompression, rapidly becomes more marked

for a few minutes after the first signs are noted, and then soon begins to mend, so that there is marked improvement in about half an hour, and by next day the animal is found quite well. This form of paralysis chiefly involves the hind legs (16 out of 19 cases).

3. *Pain.* In some cases the animals have shown signs of acute pain by urgent bleating and continual restlessness. Bleating in goats after decompression is usually a sign of distress such as is produced by cardiac and respiratory embarrassment and is often present in fatal cases. In other instances animals showing only severe bends bleat in a most distressing manner and are evidently in acute pain: at the same time they may gnaw at some part of their body (such as the testicles) as if localising the origin of the pain. In animals which have recovered, we have not had any instance where these signs persisted for more than 10 or 15 minutes.

4. *Permanent paralyses.* The onset is usually immediately after decompression, the condition is complete from the first and for at least several days there are no signs of improvement. In a few cases the first paralysis has passed off (to all appearances completely) in two or three hours and the animal has been found next morning to be again paralysed. This second paralysis is permanent. A similar history has often been noted in human cases. In 15 cases out of 16 the condition has been a paraplegia, and in one all four legs were affected more or less. In some there has been retention of urine, and one animal had to be killed on account of acute distension of the stomach which came on some 20 hours after the onset of the paraplegia. In the most severe cases the animals have been killed; others have however soon begun to mend and have lived for some months with a slight spastic paralysis of the hind legs.

5. A fair number of cases have occurred where the animal has been obviously ill, but in which it has been impossible to identify any definite local symptoms or any definite dyspnoea. The goat may lie down, refuse to move or to be tempted with corn (of which goats are inordinately fond), sometimes lying extended on the side, sometimes hurriedly rising, walking a few steps and then lying down again. On two occasions the most probable interpretation of the symptoms was that the animal was blind. The goat may run wildly about instead of becoming very apathetic and depressed. These and other such symptoms are on the whole somewhat persistent and the animal is often dull and poorly the next day. In one case (XXV A) the goat showed little but a marked apathy and distaste for food, but died 16 hours later.

6. *Dyspnoea* is usually the precursor of (7) *death* and only a minority of goats survived after showing clear dyspnoea. In these cases the condition has rapidly improved; more commonly however it progressively increases till the animal is moribund, when it is replaced by irregular, faint, gasping respiration. The mucous membranes become livid and pale and the animal lies for a short time unconscious before respiration stops. The heart continues to beat regularly throughout and the rate is not apparently much altered. Only on one occasion have we been able to hear gurgling in the heart on auscultation: it was then audible at some distance. Death ensues at varying periods after decompression; with very severe experiments (*e.g.* 100 lbs.: 1 hour: 1 minute)[1] it may follow in five or ten minutes: with more moderate conditions it is delayed for 20 or 30 minutes, or rarely for two or three hours: on three occasions it has followed still later, up to 40 hours. The delay in the onset of the first symptoms is often most striking; the animal may appear quite normal for as long as 10 or 15 minutes, dyspnoea then appears, the goat falls down helpless and in another 15 minutes is dead.

8. *Mechanical symptoms* are not important. We have not been able to satisfy ourselves that goats ever suffer materially during compression from the ear troubles which are so common in men. Abdominal distension is occasionally extreme, but the animal soon empties its distended stomach and seems to be little inconvenienced[2].

Our index throughout has been the presence of symptoms, not the presence of bubbles. Anticipating here a later section (p. 410) we may say we are in entire agreement with the view which attributes most of the severe symptoms of caisson disease to local or general blocking of the circulation by bubbles of gas. One might suppose in consequence that the incidence of severe symptoms, especially of paralyses, would be of a haphazard kind, since they would be to a large extent dependent on the chance distribution of bubbles by the blood stream. Some support for this view is perhaps to be found in the records of caisson workers given by von Schrötter; as far as can be ascertained from the details given, the cases of paralysis and dyspnoea were distributed through the whole range of pressure experienced by the men in about the same proportion to the total number of illnesses of all kinds, which latter increased greatly as the pressure became higher. It should however be noted that the range of pressure was small (up to 2·4 atmospheres positive), and the general experience of caisson works as well as our own experiments with animals are distinctly at variance with these results. We have noted only two instances of what may be called "chance incidence" of paralysis: (*a*) goat XV (Series II) had a paraplegia after 15 minutes' exposure at 75 lbs. and decompression in 30 minutes uniformly, and afterwards lived for some time, passing through much more severe experiments without symptoms, and eventually being killed with some difficulty by 75 lbs: 2 hours: 1 min.: (*b*) goat XII A (Series III) after 45 lbs.: 1 hour: 10 min. uniform, had apparently very severe bends; it did not however recover in the usual way and became partially paraplegic, subsequently passing through many comparatively severe experiments without symptoms. The tables of our results seem to show quite clearly that as the conditions of experiment become more searching, not only does the frequency of symptoms increase but the proportion of severe to total symptoms becomes much greater.

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[1] *i.e.* pressure 100 lbs. positive; exposure for 1 hour; decompression in 1 minute.

[2] Post-mortem experience shows that the stomach alone is distended, not the bowels.

It is necessary for comparative purposes to form some idea of the relative importance of these different symptoms, and to consider how far they may be classed as relatively dangerous or comparatively negligible. "Bends" are clearly a slight symptom; there is abundant evidence both in goats and men that their occurrence is no indication of urgent danger to life. At the other end of the scale we have death. Dyspnoea is not far removed in significance from death, and lasting paralyses are somewhat less serious than dyspnoea. Next in order come pain and those indeterminate conditions which we have grouped as "indefinite and various general": these may be followed by death and are much more indicative of danger than bends. Temporary paralyses are not so important and we are inclined to the view that they are not much more dangerous than bad bends. This classification is based for the most part on our experience as to the kind of experiment with which each group of symptoms is commonly associated, and the way in which the different groups are associated together in the same experiment. The individual variability of the animals introduces many difficulties, but it is certain that the more severe the conditions of pressure, exposure and decompression, the more likely it is that the animals will suffer from symptoms which we have classed as severe.

Immunity to symptoms. There is not the slighest ground, either theoretical or experimental, for supposing that animals or men, as the result of repeated exposure to compressed air, acquire any immunity to the formation of bubbles within their persons. It must be remembered in this connection that the susceptible individuals become eliminated, so that those who have been through many decompressions necessarily show more than average resistance. The matter is not so clear with regard to the exhibition of symptoms resulting from such bubbling. We have a certain amount of evidence, too vague to be detailed, that some goats show slight bends rather more easily in their first few experiences, and it is not difficult to imagine that they might grow to neglect altogether those bubbles which evidently cause them no very great inconvenience at any time. With severe symptoms it is of course different: no one can suppose that a goat acquires immunity to extensive pulmonary air emboli or to infarction of the spinal cord.

TABLE V.

Series I. June—July 1906. Pressure 75 lbs. positive (=6 atmospheres absolute). Compression in 39—41 minutes. The details of the stage decompressions are shown in Table IX.

Decompression minutes		40—50 uniform			40–50 stages		
Actual exposure minutes		12	15	30	12	15	30
No. of goat	Sex						
I	M	0					
Pa	M	0	bends	0		0	0
XIII	F	bends					
XVIII	F	bends + indefinite[1]	0	0			0
XXI	F	0+0[1]	bends				
XXII	F	0	bends				
X	F		bends	bends			0
XVI			bends	bends		0	
XX	F		bends	bends	bends		
XXIV	M		0	bends		bends	0
XXVII	F		0			0	0
XXIX	F		0	bends, dyspnoea		0	
XXX			bends			bends	
XXXII			bends				
III	M			0			0
IV	M			0			bends
VI	M			0			0
XXVI	M			bends			bends + bends[1]
XXVIII	F			bad bends			
VIII	M				0		
XIV					bends		
XIX					bends		
II	M						bends
XXV	M						bad bends

[1] Each of these experiments was repeated upon the same animal: both results are shown. XVIII was generally uneasy, lay down, nothing definite.

The details of the decompressions are shown in Table IX.

Actual exposure mins.			1	3	15					30			60		120[17]			240[17]	
Decompression mins. No. of goat	Sex	Weight kilos	1	10 uniform	10 uniform	31 stages	31 stages	31 uniform	31 uniform	31 stages	31 stages	31 uniform	31 stages	31 stages	31 stages	70 stages	70 uniform	31 stages	31 uniform
1	M	18·3		0		0	bad bends	0	0	bends	0		bends	0	bad bends	0	bends		
2	M	16·2	0			0	bends	sl. bends	paraplegia[5]	temp. paral.			0			bends	paraplegia[6]	bad bends, pain bends	
X	M	16·4	0		bad bends[1]	0	0	0	0	bends	0		0	0	bad bends[1]	bends	0		temp. paral. died[11]
XI	F	17·1			bends	0	0	0	0	0	0	bad bends	bends	0	bad bends	0	bends		
XII	M	19·8			died[7]														
XIII	M	17·8			0	0	0	0	0	0	0		bends	0	bends	0	0	0	
XIV	F	18·6		0		0	[14]	bends	bends										
XV[12]	M	16·8	0		0	sl. bends[18]	0	paraplegia[4]	0	bends			0			bends	bends	0	bad bends
XVI	M	10·7	0		bends	0[18]	0	bends	0	bends	temp. paral.	bends	0	0	bends	0	bends	temp. paral.	
XVII	F	24·8				0[18]	0	0	0	bends			bends, dyspnoea			0	temp. paral.		
XVIII	F	26·3	0			0	0	bends	0	0	bends	bends	0	obscure[3]	bad bends	0	0	bends	
XIX	F	15·0				0	0	sl. bends	0	bends	0	bends	0	0	bends	0	bends		
XX	F	15·0				0	0	0	? bends	0	0	0	0	temp. paral.	bends	0	slight bends	died[10]	
XXI[15]	F	29·7				0	bends	obscure[2]	bends	bad bends						bends, dyspnoea			
XXII	F	19·0				sl. bends	0	0	? bends	0					died[9]				
XXIII	F	21·4				0		bends	died[8]										
XXIV[16]	M	35 about																	
XXV[13]	M	21·5	0		bends	0	0	bends	sl. bends	0		temp. paral.	0			bends	bends	bends	bad bends
XXVI	F	17·5				0	0	0	0				bad bends						
XXVII	F	12·0				0	0	bends	0	temp. paral.			0			0	0		

[1] Animal also lay down and seemed generally ill: no dyspnoea. [2] Lay down, grunted, seemed quite ill: no definite bends or dyspnoea. [3] Lay down and refused to move: no definite local symptoms. [4] Could walk with feet dragging in 6 days; nearly well when killed 9 weeks later. [5] Well in 9 days. [6] Almost well in 48 hours. [7] Died in 15 minutes. [8] Died in 30 minutes. [9] Died in 76 minutes: bad bends, dyspnoea, no paralysis. [10] Pain, paralysis, dyspnoea; died 43 minutes. [11] Dyspnoea; died in 26 minutes. [12] 72 lbs., 4 hours, 40 seconds; convulsions, dyspnoea, died 20 minutes. [13] 75 lbs., 2 hours, 45 seconds; bends, convulsions, dyspnoea, died 17 minutes. [14] Injured shoulder: killed immediately after decompression: see p. 412. [15] 75 lbs., 1 hour, 1¾ minutes; died in 10 minutes without dyspnoea or any symptoms except collapse. [16] 80 lbs., 2 hours, 10 minutes uniform; bends, paralysis, dyspnoea; died in 12 minutes. [17] In some of these experiments slow compression in 39 minutes was used, in which case the actual exposure was reduced by half the time of compression. [18] A somewhat different decompression was used in this experiment, viz. 75 lbs. to 17 lbs. in 5 minutes, wait 5 minutes, then 5 minutes at 13 lbs., 10 minutes at 9 lbs., and 10 minutes at 4 lbs.

TABLE VII.

Series III. February to June 1907. Pressure 45 lbs. positive (4 atmospheres absolute). The details of the stage decompressions are shown in Table IX.

Exposure minutes			15	30	45		60				90	120				240			480
Decompression minutes . .			2	2	30 stages	30 stages	1[1]	10 uniform	30 stages	52 stages	30 stages	1	10 uniform	30 stages	57 stages	10 uniform	30 stages	62 stages	10 uniform
No. of goat	Sex	Weight kilos																	
3	M	24·3	0	0	0	0	0	bad bends	bends	0		0	0		0	0		bends	0
4	M	19·6	0	0	0	0		0	0	0		paraplegia[4]	0	0	0	0	0	0	0
X A	M	17·0	0	0	0	0	bends	0	sl. bends	0		temp. paral.	temp. paral.	0	0	bends	0	0	temp. paral.
XI A	M	20·0	0	bends	0	0	0	0	0	0		0	bends	0	0	0	0	0	0
XII A	F	19·2	0	0				paraplegia[2]			0	0	0	0	0	0	0	bends	temp. paral.
XIII A[12]	F	28·2	0	0				bends	bends	0	bad bends		bends dyspnoea paraplegia[7]	0	0		0	0	
XIV A[8]	M	16·4	0	0	0	0		bends	0	sl. bends					0			bends	
XV A	F	15·5	0	0			0	0	0	0	obscure[3]	paraplegia[5]	bends	bends	0	bends	bends	bends	bends
XVI A	M	21·1	0	bends	0	0		bends	0	0		paraplegia[6]	0	0	0	temp. paral.	0	0	0
XVII A[9]	F	26·8	0	0					0	0	0			0	0		0	0	
XVIII A[11]	F	32·0	0	0			0	0	0	0	0	0	0	0	0	bends	0	0	bends
XIX A	M	22·4	bends	0	0	0	0	0	bends	sl. bends		bends	bends	0	bends	0	0	0	0
XX A[10]	F	19·8	0	0					0	0	0			0	0		0	bends	
XXI A	F	16·0	0	bends			0	bends	0	bends	bends	bad bends	bends	0	bends	bends	bends	bends	bends
XXIII A	F	26·3	0	0				0			0		0	0	0	0	0	0	0

[1] Six other animals had this experience also, of which 7, XXVII A, and XXIX A had bends, and XXIV A, XXX A and 9 showed no symptoms. [2] Never so completely paralysed that it could not walk, but no improvement occurred during 21 weeks. [3] Bleating, seemed lost and walked into objects as if blind: well in 2 hours. [4] Acute distension of stomach developed next day; goat killed. [5] Killed next day. [6] Hind legs and, to a less degree, fore legs also: could walk in 3 days; killed 15 days when much improved. [7] Killed 3 days. [8] Broke leg, killed. [9] Died of pneumonia: no reason to connect this with decompression. [10] Died, cause unknown. [11] 75 lbs., 3 hours, 58 seconds: died 12 minutes without showing dyspnoea or any symptoms except collapse. [12] Apparently elderly.

Series IV. January to June 1907.

Pressure lbs. positive			75													75	51	45	39	30	25	20
Exposure minutes			3	6	10	15	½	15	30		60	120		180		180	180	120	120	60	240	240
Decompression minutes			1	1	¾	1	60 uniform	90 uniform	68 stages	68 uniform	86 stages	92 stages	100 uniform	134 stages	134 uniform	75 to 24 in 1½ mins., wait 1 hr., total 118 or 131 (†)[1]	4	to −6 lbs. in 6 mins.[7]	to −6 lbs. in 6½ mins.[7]	10 uniform	2	2
No. of goat	Sex	Weight kilos[2]																				
3	M	19·2		0	0	bends			0	0		0	0	0	bends					0	0	0
4	M	20·0						0				0	0							0	0	0
X A	M	17·8		0	paraplegia[11]			0	0	bends		0	temp. paral.	0						0	0	0
XI A	M	23·0		0	0	0		0	0	0		0	0	0	sl. bends					0	0	0
XII A	F	19·2		0	0	0						0	0					bends		bends	0	0
XIII A	F	28·2						0				temp. paral.	paral., dyspnoea							0	0	0
XIV A	M	16·4						0														
XV A	F	15·5						bends	0	bends		0	0	0						bends	0	0
XVI A	M	22·6						0				0	bad bends					died[6]		0	0	0
XVII A	F	26·8						0													0	0
XVIII A	F	32·0							0	0		0	0	bends	bends					0	0	0
XIX A	M	25·3						0	0	bends		bends	bends	0	? bends					bends	0	0
XX A	F	19·8						bends				0	0							0	0	0
XXI A	F	16·0		0	0	bends		bends	0	bends		0	0	0	0					bends	0	0
XXIII A	F	26·3						0				0	0					paraplegia[12]		0	0	0
XXIV A	F	14·4	0		0	temp. paral.	0		0	bends	0	bends	temp. paral.	0		? bends	bends, dyspnoea		0	0	0	0
7	M[14]	19·0	0				0		0	0				bends	bends	† bends	bends		dyspnoea	1	bends	0
XXV A	M	14·6										0	died[4]									0
XXVI A	M	14·4	0								bends	0	0			0	0				0	
XXVII A	F	27·6	0	0	0	died[3]	0		0	bends	0	0	bad bends	0		0	bad bends[13]			0	bends	0
XXVIII A	F	21·1									0	bends	pain[9], temp. paral.	0	0	bad bends	died[14]				0	
XXIX A	M	18·5					0		0	bends	0	0	pain, dyspnoea	0	0	0	indefinite[10]		bends, dyspnoea	0	0	0
XXX A	M	20·6					0		0	0						† 0	0		bends, dyspnoea	0	0	0
9	F	26·6	obscure[8]				0		0	0				0	0	† bends	bends, dyspnoea			0	0	bends
XXXII A[15]	F	22·0					bends									† 0	died[5]			0	0	0
XXVII	F	12·0					0		0	0				0	0	† 0	bends					

[1] None of the animals in these two experiments showed any symptoms while watched for 1 hour at 24 lbs. and for ½ hour at 17 lbs. The results in the Table are those observed after final decompression. [2] Some of these weights differ from those in Series III, Table VII: several of the animals became somewhat thinner in the later part of the experiments. [3] Bleated, some convulsions, fore and hind legs paralysed, a little dyspnoea; found dead next morning; no bubbles. [4] Cried out, lay down, refused to move, evidently very ill but no local symptoms or dyspnoea; died between 17½ and 18 hours later; bubbles in heart. [5] Bleating, dyspnoea, died 27 minutes. [6] Paraplegia, dyspnoea, died 190 minutes after decompression and 130 minutes after returning to atmospheric pressure. [7] In these experiments the pressure fell to atmospheric pressure in 30—35 seconds, the thermal effect then produced a delay of about 2 minutes when the pressure began to fall further, reaching −6 lbs. in about 4 minutes more: decompression was therefore in rough stages. The animals were maintained at −6 lbs. for 1 hour. [8] No symptoms for 18 minutes, then cried out, was moderately convulsed and right fore leg appeared paralysed, no dyspnoea, seemed very ill for about 10 minutes but then recovered and showed no symptoms later. [9] Constant nystagmus also noted lasting about ¼ hour. [10] Lay down, evidently ill, no definite symptoms. [11] No improvement, killed 4 days. [12] No improvement, killed 6 days. [13] On a second trial again showed severe bends. [14] Castrated, of male habit. [14] Paralysis, nystagmus, no apparent dyspnoea, died 24 minutes. [15] An aged animal.

6. *Results of goat experiments.*

The detailed results of the experiments on goats are set out in the accompanying tables. With the exception of those in Series I, these tables contain nearly all the experiments which were made. Read vertically the columns give the results of the different combinations of pressure, exposure and decompression: the records of individual goats can at the same time be read on the horizontal columns. The tables give however no indication of the chronological sequence of events. In Series I only a few experiments are given; the series actually comprised 164 experiments on 34 animals, but the procedures adopted were, through ignorance, so ill-devised that no very definite results were obtained, though we gained information which enabled us to devise more satisfactory experiments subsequently. We have therefore extracted from Series I only a few results which illustrate the difference between stage and uniform decompression. The four series roughly represent four batches of goats, except that the animals of Series IV are the remnant of Series III with the addition of a further small herd. When reference is made to individual goats of Series I, the series is noted; otherwise the goats of Series III and IV are distinguished by "A": this does not apply to hornless animals which are specified by Arabic instead of Roman numerals, no two goats having the same number.

TABLE IX. *Showing the decompressions of goats from* 75 *lbs. and* 45 *lbs. Times given in minutes, one minute being occupied in each drop after the first.*

	Decompressions from 75 lbs. +								Decompressions from 45 lbs. +			
Series	I	II	II	II	IV	IV	IV	IV	III	III	III	III
Columns..	4, 5, 6	4, 5, 8, 9, 11,12,13,16	4 (part)	14	12	7	10	9	3, 4, 7, 9 12, 15	8	13	16
First drop in	4	4	5	2	3	3	3	4	1	2	2	2
Wait at 32							3					
,, 27	5	2		7	15	4	9	2				
,, 22½	4			5	19	4	14	4				
,, 18	4	2	5	5	19	9	14	16		4	9	14
,, 14	4	4	4	10	24	9	14	16	3	14	14	14
,, 9	4 or 9	4	9	15	24	14	14	19	9	14	14	14
,, 4½	10 or 11	10	9	20	24	19	14	19	14	14	14	14
Total time	41 or 50	31	36	70	134	68	92	86	30	52	57	62

In the next table (Table X) the results are condensed and grouped in a simpler way, and one or two more experiments are given from Series I.

TABLE X.

Pressure lbs. positive	Compression minutes	Actual exposure minutes	Decompression minutes	No. of goats	No symptoms		Bends					Temporary paralysis	Various indefinite	Paraplegia	Dyspnoea	Total severe symptoms	Death
					Number	Percent.	?	Slight	'Bends'	Bad	Total						
75	6	½	60 un.	8	7	87			1		1					0	0
		1	1	6	6	100					0					0	0
		3	1	5	4	80					0		1			1	0
		3	10 un.	2	2	100					0					0	0
		6	1	6	6	100					0					0	0
		10	1	7	6	86					0			1		1	0
		15	1	6	2	33	1		1		2	1				1	1
		15	10 un.	7	2	29			3		3		1			1	1
		15	31 st.	34	29	85		2	2	1	5					0	0
		15	31 un.	36	19	53	2	3	8		13		1	2		3	1
		15	90 un.	12	9	75			3		3						
		30	31 st.	23	12	52			7	1	8	3				3	0
		30	31 un.	6	1	17			3	1	4	1				1	0
		30	68 st.	14	14	100					0					0	0
		30	68 un.	14	7	50			7		7					0	0
		60	31 st.	22	15	68			3	1	4	1	1		1	3	0
		120	31 st.	9	0	0			4	3	7		1			1	1
		120	70 st.	14	9	64			4		4				1	1	0
		120	70 un.	13	4	31		1	6		7	1		1		2	0
		120	92 st.	19	15	79			3		3	1				1	0
		120	100 un.	19	10	53			1	2	3	2	1		2	5	1
		180	134 st.	14	12	86			2		2					0	0
		180	134 un.	10	5	50	1	1	3		5					0	0
		240	31 st.	8	2	25			3	1	4	1				1	1
		240	31 un.	4	0	0				2	2	1				1	1
51	6	180	4	10	2	20			2	1	3		1		2	3	2
45	6	15	2	15	14	93			1		1					0	0
		30	2	15	12	80			3		3					0	0
		45	30 st.	14	14	100					0					0	0
		60	1	13	10	77			4		4					0	0
		60	10 un.	13	7	54			4	1	5			1		1	0
		60	30 st.	13	9	69		1	3		4					0	0
		60	52 st.	13	10	77		2	1		3					0	0
		90	30 st.	8	5	62			1	1	2		1			1	0
		120	1	10	4	40			1	1	2	1		3		4	0
		120	10 un.	12	6	50			4		4	1			1	2	0
		120	30 st.	13	12	92			1		1					0	0
		120	57 st.	15	13	87			2		2					0	0
		240	10 un.	11	6	55			4		4	1				1	0
		240	30 st.	13	11	85			2		2					0	0
		240	62 st.	15	9	60			6		6					0	0
		480	10 un.	11	6	55			3		3	2				2	0
30	6	60	10 un.	19	15	79			4		4					0	0
25		240	2	23	21	91			2		2					0	0
20		240	2	22	21	95			1		1					0	0
75	39	1	7 un.	8	7	87					0			1		1	0
		10	10 un.	4	3	75					0					0	1
		12	45 st.	4	1	25			3		3					0	0
		12	45 un.	6	4	67			2		2					0	0
		15	45 st.	6	4	67			2		2					0	0
		15	45 un.	12	4	33			8		8					0	0
		30	45 st.	11	7	64			3	1	4					0	0
		30	45 un.	12	5	42			5	1	6				1	1	0
		30	10 un.	4	0	0					0					0	4
		30	10 un.[1]	4	4	100					0					0	0
		30	10 un.[2]	4	0	0			2		2			1		1	1
		60	75 un.	4	2	50			1		1					0	1

[1] Recompressed at once to 15 lbs. for 32 minutes.

[2] Recompressed to 15 lbs. for 37 minutes 18 minutes after decompression.

un. = uniform decompression : st. = decompression by stages.

The following tables give in the simplest form the experimental evidence on certain points which are of especial importance.

(I) *Experiments showing that a certain minimum pressure is required to give symptoms in goats, and that the results vary with the pressure.*

TABLE XI.

Pressure in lbs. positive	Exposure in minutes	Decompression in minutes	No. of goats	No symptoms	Bends	Severe symptoms	Death
20	240	2	22	21	1	0	0
25	240	2	23	21	2	0	0
30	60	10 uniform	19	15	4	0	0
45	60	10 ,,	11	7	3	1	0
60[1]	45	15 ,,	4	1	3	0	0
75	15	31 ,,	36	19	13	3	1
75[2]	50	10 ,,	4	0	0	0	4

[1] Experiment in Series I: compression 30 minutes, exposure 30 minutes.

[2] Series I: compression 40 minutes, exposure 30 minutes.

These experiments show that the effects become more severe as the pressure increases although the duration of exposure was at the same time diminished and the duration of decompression increased. It was necessary to arrange the experiments in this way to prevent an inconvenient mortality among the animals.

(II) *Experiments showing that the duration of exposure to high pressures is of great importance.*

TABLE XII.

Pressure 75 lbs. positive, reached in 6 minutes.

Exposure in minutes	Decompression in minutes	No. of goats	No symptoms	Bends	Severe symptoms	Death
1	1	6	6	0	0	0
3	1	5	4	0	1	0
6	1	6	6	0	0	0
10	1	7	6	0	1	0
15	10 uniform	7	2	3	1	1
15	31 stages	34	29	5	0	0
30	31 ,,	23	12	8	3	0
60	31 ,,	22	15	4	3	0
120	31 ,,	9	0	7	1	1
240	31 ,,	8	2	4	1	1

These experiments show that goats have taken up enough air in 15 minutes to give severe symptoms on decompression in 10 minutes, while, if the exposure is less than 10 minutes, nearly all the animals escape, even with sudden decompression. Note too that with short exposures and rapid decompressions such symptoms as appear are more frequently severe, and that bends are proportionately less common than with longer exposures and slower decompressions. Beyond 15 minutes exposure the results are somewhat irregular, but on the whole there is a progressive increase of bad symptoms up to two hours exposure. The results after four hours exposure are about the same, but the animals used (see Table VI, Series II) were to a large extent selected by previous experiments, so that it would appear that goats are practically saturated in about three hours[1].

TABLE XIII.

Pressure 45 lbs. positive.

Exposure in minutes	Decompression in minutes	No. of goats	No symptoms	Bends	Severe symptoms	Death
15	1	15	14	1	0	0
30	1	15	12	3	0	0
60	1	14	10	4	0	0
120	1	10	4	2	4	0
60	10 uniform	11	7	3	1	0
120	10 ,,	11	6	4	1	0
240	10 ,,	11	6	4	1	0
460	10 ,,	11	6	3	2	0

These figures show that with a duration of exposure up to about three quarters of an hour, no severe symptoms follow even sudden decompression. The series with sudden decompression shows that the results after two hours are much worse than after one hour. This is not clear from the series with 10 minutes decompression, which, however, show that the results do not become distinctly worse even after

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[1] The following figures have been compiled from the records of Heller, Mager and von Schrötter as illustrating the saturation time for dogs of about 10 (?) kilos. The corresponding data for other animals do not seem to have been determined. Pressure 62—69 lbs., compression in 5—16 minutes, decompression ½ to 1 minute.

Exposure minutes	Number of experiments	No symptoms	Mild paralysis and bends	Lasting paralysis	Paralysis and asphyxia	Asphyxia
Less than 10	1	1	0	0	0	0
10—29	6	5	1	0	0	0
30—59	12	0	6	2	3	1 (lived)
60—120	19	2	2	2	3	10

Four of the group "paralysis and asphyxia" died, and the other two would probably have died if they had not been killed. All but one in the "asphyxia" group died, but in none of the rest was the decompression immediately fatal. These results seem to show pretty clearly that dogs require more than an hour to become saturated. It is strange that the authors conclude (*Luftdruckerkrankungen*, p. 806) that saturation is so far complete in about 38 minutes *in man* that no further intake of nitrogen is of any practical importance.

eight hours exposure. Note that of the nine severe symptoms, five were temporary and four permanent paralyses: only one case of dyspnoea was seen in the whole of the experiments at 45 lbs. (Series III) and the one case of severe illness of obscure nature was suggestive of temporary local cerebral anaemia. At 75 lbs., out of 26 severe cases, four had dyspnoea, four permanent and 12 temporary paralysis, and six indefinite: seven died.

(III) *Experiments to show that the duration of decompression is of great importance.*

TABLE XIV.

Pressure 75 lbs. positive, reached in 6 minutes.

Exposure	Decompression	No. of goats	No symptoms	Bends	Severe symptoms	Death
15	10 uniform	7	2	3	1	1
15	31 ,,	36	19	13	3	1
15	90 ,,	12	9	3	0	0
30	31 stages	23	12	8	3	0
30	68 ,,	14	14	0	0	0
120	31 ,,	9	0	7	1	1
120	92 ,,	19	15	3	1	0

(IV) *Experiments to show that the absolute range of pressure through which decompression occurs may be of less importance than the relative range of absolute pressure.*

TABLE XV.

Pressure in lbs. +	Exposure in minutes	Decompression to lbs.	Fall of pressure in lbs.	Relative reduction of absolute pressure	Duration of decompression in minutes	No. of goats	No symptoms	Bends	Severe symptoms	Death
75	180	+24	51	2·3 : 1	1½	10	10	0 [1]	0	0
51	180	0	51	4·4 : 1	4	10	2	3	3	2
45	120	−6	51	6·7 : 1	6	3	0	1	1	1
39	120	−6	45	6·0 : 1	6	4	1	0	3	0
45	120	0	45	4·0 : 1	1	10	4	2	4	0

[1] There were three cases of bends at the ultimate end of a two hours' decompression.

A sudden drop of about 50 lbs. from 75 lbs. positive to 27 or 24 lbs. positive has been made about 200 times altogether in the course of these experiments without producing any symptoms, and about two-thirds of the animals showed no symptoms at the end of the stage decompression. The animals were however only left a short time at 27 lbs. before proceeding with the further decompression. In the present series the animals were left for one hour at 24 lbs. and watched very carefully, and afterwards suddenly decompressed to 17 lbs. and again observed for half an hour. The same goats were subsequently dropped suddenly from + 51 lbs. to atmospheric pressure with very disastrous results, and a drop of 51 lbs. from + 45 lbs. to − 6 lbs. was even worse. The details of these experiments are given in Appendix III. Owing to the cooling effect of rapid decompression, the falls from + 45 and + 39 to − 6 lbs. were interrupted by a delay of about two minutes at atmospheric pressure so that they were in a rough way stage decompressions.

(V) *Experiments showing the importance of the mode and spacing of decompression.*

The next table shows in brief the results of seven groups of experiments undertaken with the purpose of directly testing the results of stage decompression in comparison with those of uniform decompression in the same total time. The only exceptions to the parallelism of the experimental conditions are (1) in group ζ the time of uniform decompression was extended from 92 to 100 minutes in order that it might correspond to the supposed safe rate of 20 minutes an atmosphere[1]; and (2) in group β stage decompression, three animals were decompressed by stages in an abnormal way (see Table VI and note, Series II); since these stages were certainly not more favourable to the animals than those used for the rest of the group, we have included the results.

In considering these results it must be clearly understood that the stage decompressions used were not in most cases intended to be safe for the particular exposure to which they were attached. The only two groups which were intended to be safe (δ and η) gave fairly satisfactory results; with 30 minutes exposure (+ 6 minutes compression) at 75 lbs. and 68 minutes stage decompression, we obtained no illnesses in 14 goats, and with three hours exposure and two and a quarter hours stage decompression only two cases of bends in the same number of animals. For comparative purposes it was desirable that the stage decompressions should produce symptoms of some kind, and they were intentionally designed so to do in so far as our knowledge allowed[2].

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[1] The details of the experiments in this group are given in Appendix III.

[2] The stage decompressions from 45 lbs. pressure are likewise all shorter than what we calculate to be safe. The stoppages are also imperfectly spaced. The proper spacing and duration of stoppages could not be calculated till the results of the experiments were known, and we realised the extreme slowness of saturation and desaturation.

TABLE XVI. *Showing the comparison between the results of stage and uniform decompression. Pressure 75 lbs. positive.*

A. *All experiments.*

							Bends										
Exposure minutes[1]	Decompression, minutes	Decompression, method	Number of animals	Number of decompressions	No symptoms	Per cent.	Doubtful	Slight	Bends	Bad	Total	Temporary paralysis	Various general	Paraplegia	Dyspnoea	Total severe	Death
12–30	45	stages	18	22	12	55			9	1	10					0	
15	31	,,	18	34	29	85		2	2	1	5					0	
30	31	,,	15	23	12	52			7	1	8	3				3	
30	68	,,	14	14	14	100					0					0	
120	70	,,	14	14	9	64			4		4				1	1	
120	92	,,	19	19	15	79			3		3	1				1	
180	134	,,	14	14	12	86			2		2					0	
otal				140	103	74	0	2	27	3	32	4	0	0	1	5	0
12–30	45	uniform	19	32	14	44			15	1	16		1		1	2	
15	31	,,	18	36	19	53	2	3	8		13		1	2		3	1
30	31	,,	6	6	1	17			3	1	4	1				1	
30	68	,,	14	14	7	50			7		7					0	
120	70	,,	13	13	4	31		1	6		7	1		1		2	
120	100	,,	19	19	10	53			1	2	3	2	1		2	5	1
180	134	,,	10	10	5	50	1	1	3		5					0	
otal				130	60	46	3	5	43	4	55	4	3	3	3	13	2

B. *Experiments on identical animals.*

							Bends										
Exposure minutes[1]	Decompression, minutes	Decompression, method	Number of animals	Number of decompressions	No symptoms	Per cent.	Doubtful	Slight	Bends	Bad	Total	Temporary paralysis	Various general	Paraplegia	Dyspnoea	Total severe	Death
15	31	stages	18	34	29	85		2	2	1	5					0	
30	31	,,	6	6	4	67			2		2					0	
30	68	,,	14	14	14	100					0					0	
120	70	,,	13	13	9	69			4		4					0	
120	92	,,	19	19	15	79			3		3	1				1	
180	134	,,	10	10	8	80			2		2					0	
otal				96	79	82	0	2	13	1	16	1	0	0	0	1	0
15	31	uniform	18	36	19	53	2	3	8		13		1	2		3	1
30	31	,,	6	6	1	17			3	1	4	1				1	
30	68	,,	14	14	7	50			7		7					0	
120	70	,,	13	13	4	31		1	6		7	1		1		2	
120	100	,,	19	19	10	53			1	2	3	2	1		2	5	1
180	134	,,	10	10	5	50	1	1	3		5					0	
otal				94	46	49	3	5	28	3	39	4	2	3	2	11	2

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[1] Group *a* compressed in 39 minutes; the rest in 6 minutes or, with long exposures, in 39 minutes and half the time of compression deducted from the actual time of exposure.

The results are given in two forms: (*A*) shows the fate of all the animals tested to obtain the direct comparison between stage and uniform decompression, while in (*B*) the figures are confined to the effects (in the same experiments) on animals which were exposed to both stage and uniform decompression in each group. This emendation removes the only very severe symptom and three out of four of the temporary paralyses caused by stage decompression. Goat XXI (Series II) was advancing in pregnancy and, after having nearly died as the result of quick stage decompression, was excluded from the experimental troupe; the effect of the corresponding uniform decompression on this animal can therefore be only surmised. The effects of 31 minutes stage decompression after 30 minutes exposure were so bad that, not wishing at this stage to risk losing any animals, the parallel experiment with uniform decompression was limited to the more resistant animals. In group β (*B*) two of the animals were only decompressed once by stages. One had died from uniform decompression, and the other had broken a leg and had to be killed.

The figures show that the ratio of animals showing no symptoms with stage decompression to those escaping after uniform decompression in the same total time is about eight to five. Be it noted too that the difference between the two methods is in the same sense, *i.e.* in favour of stage decompression, in each of the seven groups, including group α (Series I) where the stages were less well arranged than afterwards. The difference between the two methods appears still more strikingly in the quality than in the quantity of the symptoms produced. For while but one animal had symptoms which can be called distinctly severe after stage decompression, as many as eleven were materially ill after the corresponding uniform decompressions, and one died.

This difference may perhaps obtain more definite expression if we assign numerical values to the different symptoms. Making bends = 1, temporary paralysis = 2, and so on up to death = 6, we obtain the following results, showing a ratio of nearly five to one (*B* grouping) in favour of stage decompression:—

Group	Stages	Uniform
β	5	30
γ	2	6
δ	0	7
ε	4	13
ζ	5	26
η	2	5
Total	18	87

This method is of course very rough. "Death" is worth more than six times "bends," and bends should have different values according to the sort of experiment. Bends arising from short exposures and relatively rapid decompressions (*e.g.* group β) indicate that the exposure has been long enough to allow material saturation and are very significant, while if bends show merely the extreme slowness with which the tissues in which they arise get rid of the excess gas (*e.g.* group η), they are of much less moment.

If we exclude bends, and count only the more serious symptoms, or death, the comparison becomes still more striking, the ratio being then two for stage decompression, as compared with 50 for uniform decompression.

(VI) *Experiments illustrating the difference between different kinds of animals.*

(1) Five goats (XXIV A, XXVI A, XXVII A, 7 and 9), 10 small guinea-pigs (175 to 275 gms., average 230 gms.), 9 mice (average 20 gms.), 12 small rats (average 35 gms.), 9 medium rats (average[1] 85 gms.), 8 large rats (average[1] 200 gms.), and 4 rabbits (1285, 1450, 1850, 2850 gms.) were compressed to 72 lbs. in 7 minutes and left at that pressure for 3 more minutes and decompressed in 50 seconds. Goat 9 had a curious short seizure and rolled over on the ground 10 minutes after decompression; it seemed alright immediately afterwards and showed no after effects. One small rat became paraplegic at once, and two other small rats were found dead next morning; one of these had bubbles in the heart. The rest of the animals showed no symptoms. The incidence of illness on the young rather than on the old rats is curious in view of the demonstration of the general immunity of young animals by Hill and Greenwood[2]: it was perhaps correlated with the shortness of the exposure.

(2) Twelve small rats, 13 medium rats, 8 large rats, 59 mice, 7 rabbits, 10 guinea-pigs, and 1 old hen were raised to 72 lbs. in 10 minutes, left for 1 hour and then decompressed in 50 seconds. No goats were put in since it was well established that this experience would have killed all of them. The hen and the largest rabbit (weight 2800 gms.) died in 5 minutes, and 1 guinea-pig became paraplegic in 10 minutes and died in 20 minutes. All three were extensively

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[1] The details were eaten by a goat. All the animals were about the same size.

[2] *British Medical Journal*, June 22nd, 1907, Supplement, p. 408.

bubbled; it is interesting to note that there were no bubbles in the avascular eggs of the hen. None of the other animals showed any symptoms.

(3) Five rabbits, 10 guinea-pigs, 23 mice, 10 small, 9 medium and 6 large rats were compressed to 51 lbs. in 7 minutes, left there for 2 hours 56 minutes and decompressed in 45 seconds. In similar experiments, out of 10 goats 2 died and only 2 escaped without symptoms (see above, p. 398). The largest rabbit, a very fat animal weighing 2·9 kilos, died 9 minutes after decompression: the rest showed no symptoms.

(4) Six goats (3, XI A, XII A, XXI A, XXIV A, XXVII A), 7 guinea-pigs and six rabbits were raised to 75 lbs. in 5½ minutes, left for 15 minutes and decompressed in 42 seconds. Goat XXVII A had dyspnoea and paraplegia and was found dead next morning: XXIV A had temporary paralysis of both hind legs without dyspnoea and was quite recovered in an hour: 3 and XXI A had bends, while XI A and XII A showed no symptoms. None of the small animals were affected.

(5) Seven goats (3, X A, XI A, XII A, XXI A, XXIV A, XXVII A), 7 guinea-pigs, 5 rabbits, 7 medium and 12 large rats and 37 mice were compressed to 75 lbs. in 6 minutes, left 10 minutes and decompressed in 48 seconds. Goat X A had paraplegia. The other goats and the small animals showed no symptoms.

(6) Guinea-pigs, mice and rats were compressed with ourselves to 30 lbs. in 15 minutes, and 1 guinea-pig, 1 mouse, 1 medium and 1 large rat were killed with chloroform after 33 minutes. After decompression in 26 minutes by stages, many bubbles were found in the heart and vessels of the guinea-pig, a few in the mouse's heart, a few in the great vessels of the large rat, but none in the medium sized rat.

7. *Individual variation among the experimental animals in their susceptibility to decompression symptoms.*

The variation in the individual susceptibility of different goats is very marked. The same variation has been noted constantly among both divers and caisson workers, and is apparent in most of the published animal experiments. As an example, the following figures have been extracted from the tables of experiments at 75 lbs. All four animals were males and very similar to one another in all obvious respects: two were resistant and two susceptible.

TABLE XVII.

Exposure	Decompression	XIII (17·8 kg.)	X (16·4 kg.)	2 (16·2 kg.)	XV (16·8 kg.)
15 mins.	31 mins. uniform	0	0	slight bends	paraplegia
15 ,,	31 ,, ,,	0	0	paraplegia	0
15 ,,	31 mins. stages	0	0	0	slight bends
15 ,,	31 ,, ,,	0	0	bends	0
30 ,,	31 ,, ,,	0	bends	pain, temporary paralysis	bends
60 ,,	31 ,, ,,	bends	0	0	0
120 ,,	70 mins. uniform	0	bends	paraplegia	bends
120 ,,	70 mins. stages	0	0	bends	bends

In all, therefore, goats X and XIII showed mild symptoms three times in 16 decompressions, while in the same experiments goats 2 and XV showed symptoms 11 times, and on 4 occasions these were of a severe character.

It might be supposed that this variation was only in the exhibition of symptoms, depending on individual susceptibility to pain, &c., and did not represent a variation in the amount and distribution of bubbles within the body. But post-mortem experience shows that the amount of bubbling present in two animals killed in the same experiment may be very different; and in living animals it is clear that on the whole susceptibility to bends involves susceptibility also to the more severe symptoms, which cannot be much altered by the temperament of the animal.

The complete explanation of this individual variation in susceptibility probably requires a knowledge of the details of caisson disease far beyond that which we at present possess. Data exist, however, on which the influence of several factors may be discussed.

(A) *Influence of sex.* The following table shows the sum of the results of the experiments grouped according to the sex of the animals. The groups defined as "selected" include only those experiments in which the animals examined were approximately representative: in Series II for example the figures given are the totals of those experiments in which 10 or more animals were examined, while in Series III and IV are summed only those observations which included both sexes about equally (Series III, expts. 1, 2, 5—8, 10—17, Series IV, expts. 18—20).

It would appear from this that there is no clear difference between the sexes in liability to decompression symptoms in general. The experiments suggested however that under certain circumstances there might be a marked difference in the susceptibility to death. In

Series I, of 5 deaths, 3 were in females, a distribution of fatalities corresponding to the numbers of the sexes (males 12, females 16) used, while in Series II are shown 1 death in 7 males and 4 deaths in 11 females. All these last four animals were to some degree advanced in pregnancy, and their mortality is very probably to be associated with this condition, which, in the goat, is accompanied by a marked increase in the subcutaneous and intra-abdominal fat. That the deaths in Series I did not fall more heavily upon the females is perhaps to be correlated with the fact that these experiments were made in the summer and none of the goats were found pregnant, while the autopsies of Series II showed that in the winter practically every female is pregnant.

TABLE XVIII.

	Males				Females			
	Number	Decompressions	Illnesses	Per cent.	Number	Decompressions	Illnesses	Per cent.
Series I:								
Total	12	78	25	32	16	71	35	49
Series II:								
Total	7	84	42	50	11	91	38	42
Selected	7	64	26	41	11	79	27	34
15 mins. exposures	7	28	9	32	11	42	13	31
1 and 2 hrs. exposures	7	25	11	44	8	24	10	42
Series III and IV:								
Selected	7	108	26	24	8	113	29	26
Total	26	270	93	34	35	275	102	37

Influence of size. In the same way the influence of size on susceptibility may be examined. In the next table the animals are grouped as above and below the average weight for each sex.

TABLE XIX.

	Above average weight			Below average weight		
	Decompressions	Illnesses	Per cent.	Decompressions	Illnesses	Per cent.
Series II:						
Selected	55	22	40	80	26	32·5
15 mins. exposure	27	9	33	39	9	23
1 and 2 hrs. exposure	20	9	45	26	12	46
Series III and IV:						
Selected	102	19	19	119	36	30
Total	157	41	26	199	62	31

The results are contradictory unless (which appears hardly possible) there is an essential difference between exposures to high (75 lbs.) and to low (45 lbs.) pressures. The sums of the whole show no material difference between large and small goats. Theoretically, with decompressions of moderate length such as were used in the experiments under consideration, small goats should be somewhat more susceptible than large goats with short exposures since they should saturate more quickly in proportion to their relatively greater gaseous exchange[1]. This is not borne out by the experiments, in which however the rate of decompression may not have been quick enough to bring out the difference. It is, on the other hand, obvious that the larger goats should be more susceptible after long exposures with any except very short or very long decompressions: this is confirmed by the experiments at 75 lbs. (Series II), but those at 45 lbs. (Series III) show a greater difference in the opposite sense.

In comparing the incidence according to sex with those arranged according to weight, it will be noted that in Series II the males are somewhat more susceptible though they are rather smaller, while the same experiments, arranged by weights, show that the heavier animals suffer more frequently. In Series III, in which the male group is again composed of smaller animals, the susceptibility of the sexes is equal, while the lighter animals are more susceptible if weight be taken as the criterion. The only conclusion to be drawn is that these figures do not indicate that either sex or weight was a determining factor in the incidence of decompression symptoms.

Influence of the activity of gaseous exchange. General considerations suggest rather strongly that the susceptibility would be found to vary with the activity of gaseous exchange, directly as regards short exposures and inversely as regards long exposures. In most of our experiments, especially those of Series III, the incidence of symptoms has been conditioned rather by the mode of decompression than by the duration of exposure. As a whole, then, the goats with the most active exchange should prove to be the least susceptible.

The respiration results already given have been analysed in reference to this point: the results are variable and inconclusive and need not be detailed. This is perhaps not very remarkable when we consider that the animals were not grouped for the respiration experiments according to their susceptibility but by the bands into which they had been marshalled for the pressure experiments. A factor of considerable importance, which is to a large extent beyond control, is the activity of the goats at the moment. Some goats are naturally vivacious while others are almost constantly lethargic. These individual idiosyncrasies are no doubt of some moment in relation to susceptibility, but the customary habits of a group of animals may be altogether upset by an incompatible companionship in the chamber during an experiment.

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[1] The respiratory activity per unit of body weight, being proportional to the ratio of surface to mass, would of course vary but little in the goats, and would only be about a fourth greater in a goat of 15 kilos than in one of 30 kilos.

One group of measurements gave for example the results shown in the following table. The CO_2 production of each group was determined on four separate occasions under conditions similar to those obtaining in the pressure experiments.

TABLE XX.

Females.

No. of goat	Weight kilos	Expts.	Ill-nesses	Pressure lbs.	CO_2 gms. per kilo per hour
XII A	14·2	14	4	0	0·921
XV A	15·5	17	8	45	1·104
XVIII A	31·4	17	2	45	1·020
XXI A	17·0	17	11	45	0·852
XXIII A	22·0	13	0		
Average	20·0	78	25 (32 %)	—	0·974

Males.

No. of goat	Weight kilos.	Expts.	Ill-nesses	Pressure lbs.	CO_2 gms. per kilo per hour
3	19·2	15	3		
4	20·0	16	1	0	0·669
X A	17·8	17	6	45	0·697
XI A	23·0	17	2	25	0·959
XVI A	22·6	16	4	45	0·967
XIX A	25·3	17	7		
	21·3	98	23 (23 %)	—	0·823

The average size in each group is about the same, and the sex incidence for all goats of Series III is the same. The results therefore appear to show that the males are 32 % less susceptible and produce 17 % less CO_2—a result which cannot be correlated with theory.

The only experiments made with the animals grouped according to their susceptibilities gave much more rational results. Great care was taken in this series to make the conditions as nearly identical as possible in all four observations; the animals were kept in the dark and remained quite quiet throughout. The results show a CO_2 production by the susceptible animals one-sixth less than that of the non-susceptible.

Influence of blood volume. The volume of the blood was determined in 8 goats, in 7 of which the susceptibility to caisson symptoms had been ascertained. The method used was the simple one of Welcker, in which, after taking a standard sample of arterial blood, the animal is bled to death and then thoroughly washed out with salt solution. The

TABLE XXI.

Non-susceptible goats.

Animals							Respiration					
			Total		Selected						CO_2 gms. per hour	
No. of goat	Sex	Weight	Decomp.[1]	Ill	Decomp.	Ill	No. of expt.	Temp.	Bar. m.m.	Duration in hours	per kilo	per 1000 sq. cms.
4	M	18·6	15	0	10	0						
XI A	M	20·8	16	1	11	1	I	12°	776	5	0·717	1·853
XVIII A	F	30·7	14	0	10	0	III	14°	775	5	0·751	1·941
XXIII A	F	25·2	12	0	8	0						
Average		23·8	57	1 (2 %)	39	(2½ %)					0·734	1·897
Susceptible goats.												
XV A	F	15·0	15	6	10	4						
XXI A	F	15·4	15	8	10	6	II	14°	775	5	0·624	1·501
XIX A	M	21·3	15	7	10	5	IV	13°	766	5	0·704	1·693
XIII A	F	25·2	16	5	11	2						
Average		19·2	61	26 (43 %)	41	17 (41 %)					0·664	1·597

[1] These figures are given up to the date at which the respiration experiments were made. Some time elapsed before the final susceptibilities were ascertained: these were 8 % for the non-susceptible group and 45 % for the susceptible animals.

tissues were not afterwards extracted with water: the red colouring matter so obtained is so small in amount that it can have little influence on the final result, and Douglas[1] has shown that additional difficulties are thereby introduced. For purposes of calculation the specific gravity of the blood has been taken as 1050. The results and the decompression records of the goats are given in the two following tables. The figures should be read in relation to the "clean" weight, *i.e.* the crude weight less the weight of the contents of the alimentary canal, which in these animals is very considerable.

The results seem to indicate that there are two types of blood volume in goats: one about 7½ % of the clean body weight and the other about 6½ %, the first type being also associated with a higher percentage of haemoglobin. No relation between blood volume and susceptibility is apparent; thus goats 2 and XIII, both males, have identical blood volumes and differ about as widely in their susceptibility as any two goats which have come under our notice.

Conclusions. Of the four factors considered in detail, it appears therefore that age, sex and blood volume were without appreciable influence. Pregnancy and a low rate of respiratory exchange seem to favour the occurrence of symptoms.

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[1] *Journal of Physiology*, vol. XXXIII. (1906), p. 499.

TABLE XXII.

No. of goat	Sex	Whole weight kilos	Weight of contents of stomach and intestines: Total kg.	Per cent. of whole weight	Volume of blood c.c.	Mass of blood per cent. of: Whole weight	Clean weight	Haemoglobin p.c. of human standard[1]
1	M	19·9	3·8	19·1	1006	5·31	6·56	74
XIII	M	18·9	4·3	22·7	883	4·91	6·36	—
2	M	18·0	3·7	20·5	874	5·10	6·42	64
X	M	17·0	3·0	17·6	833	5·14	6·25	72
XVI	M	11·3	2·3	20·3	592	5·50	6·91	64
A	F	31·2	5·2	16·7	1874	6·31	7·57	78
XVIII	F	27·1	4·6	17·0	1395	5·41	6·47	75
XVII	F	24·4	4·0	16·4	1520	6·54	7·83	84
Average		21·0	3·9	18·8	1122	5·53	6·80	73
Average of males		17·0	3·4	20·0	838	5·19	6·50	68·5
Average of females		27·6	4·6	16·7	1596	6·09	7·29	79

[1] The red blood corpuscles of goats are very small, about 4μ in diameter (Jolly, *C. R. Soc. de Biol.*, vol. LXIII (1907), p. 210).

TABLE XXIII.

Pressure 75 lbs.

Exposure minutes	Decomp. minutes	Goat 1	2	X	XIII	XVI	XVII	XVIII
15	30 un.	0	slight bends	0	0	bends	0	slight bends
15	30 un.	0	paraplegia	0	0	0	0	0
15	30 st.	0	0	0	0	0	0	0
15	30 st.	bad bends	bends	0	0	0	0	0
30	30 st.	bends	temp. paral.	bends	0	bends	bends	0
60	30 st.	bends	0	0	bends	0	dyspnoea	0
120	70 un.	bends	paraplegia	0	0	bends	temp. paral.	0
120	70 st.	0	bends	bends	0	0	0	0
15	1	—	—	—	—	bends	—	—
15	10 un.	—	—	bad bends	0	bends	—	—
30	30 un.	—	—	—	—	bends	—	bends
30	30 st.	0	—	0	0	temp. paral.	—	bends
60	30 st.	0	—	0	0	0	—	obscure
120	30 st.	bad bends	—	bad bends	bends	bends	—	bad bends
240	30 st.	—	bad bends	bends	0	temp. paral.	—	bends
240	30 un.	—	—	temp. paral.	—	—	—	—

But there are doubtless other particulars which, alone or in combination, are of fundamental importance. Such other factors we have not yet been able to examine in detail. Fatness for example can be gauged only in the dead, and, though we have the distinct impression that the goats which die easily (*i.e.* under circumstances of pressure, exposure and decompression which cause very few severe symptoms and deaths) are fatter than those which are killed with difficulty, we have had no means of extending our observations on this head to the great majority of our animals[1]. Fatness also involves a low rate of respiratory exchange per unit body weight. There are grounds in human experience for holding that age may have an important share in the production of symptoms and Hill and Greenwood have recently shown[2] clearly that young animals (rats, rabbits and cats) are far less susceptible than adults of the same species. All the goats used by us appeared to be adult; in the two cases (XIII A, XXXII A) in which old age had obviously set in, the susceptibility seemed to be somewhat above the average, but the ages of the animals as a whole were unknown. In any case such a factor as old age must be reduced to simpler components before it can be correlated with the theory of decompression.

8. *The pathology of caisson disease in goats.*

We have hitherto dealt exclusively with the symptoms exhibited by the experimental animals rather than with the actual or possible presence of bubbles within them. We have however made a number of observations on the post-mortem appearances of goats after decompression, which may be shortly dealt with here. Most of the animals had died from caisson disease but in other instances they were killed at varying periods after decompression.

The presence of bubbles *in vivo* must be inferred from their discovery post-mortem with considerable caution. The supersaturation of the body may be such that the separation of the gas as bubbles may take place after death. There are reasons for supposing that the living body presents nothing in the way of points or surfaces on which bubbles might arise in the blood and tissues as they do upon the glass and dust in soda-water, and a remote analogy may perhaps be drawn with the relations of the vessels to the separation of fibrin *intra vitam*. Death may well alter this condition in some degree, and in any case the time factor is of importance as well as the foreign manipulation which examination involves. During life no portion of the blood remains in a supersaturated state for more than the time required for it to return from the tissues to the lungs. We have already seen that the duration of this period is probably of very great importance as regards risk of bubble formation. On cessation of the circulation the blood remains in a supersaturated state for an indefinite period. In one instance at least we have actually observed such post-mortem separation of bubbles: a rabbit was killed immediately after 75 lbs., 2 hours, 31 minutes stages, opened up at once and no bubbles found[1]; an hour later a few bubbles were found in the inferior *vena cava*. In another case bubbles in the bladder were seen to increase considerably in number and volume during the progress of the examination.

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[1] We have since examined this point by direct analysis of rats and guinea-pigs divided into susceptible and non-susceptible groups by decompression experiments. The results, which will be published in detail later, show that fatness is a very important factor in individual susceptibility to death.

[2] Meeting of Physiological Society, Nov. 1906.

The possibility of air being introduced from without into the veins must also be considered. Ewald and Kobert showed that air might be forced into the pulmonary capillaries by an increase of intra-pulmonary pressure such as may occur in severe dyspnoea. We have seen bubbles in large quantities in the meningeal veins, and in small numbers in the superficial veins of a fore-foot, under circumstances which left no reasonable doubt that they had been sucked into the vessels during the somewhat violent manipulations used in opening the skull and skinning the leg respectively.

There is not much doubt that some of our animals which showed no symptoms must have had bubbles present in the blood. Catsaras decompressed a dog in 1 minute after 2 hours exposure at 65 lbs.: it showed no symptoms and was killed 6 hours later, when fine bubbles were found in the blood. Heller, Mager and von Schrötter (pp. 790, 882) record two dogs which were killed 10 minutes after sudden decompression after 65 and 52 minutes at 15 and 18 lbs. respectively: in both cases bubbles were found in the heart[2], though there is abundant evidence to show that dogs never show any symptoms after decompression from such low pressures. In our own animals attempts were made to see bubbles in the retinal vessels during life. Though an excellent view of the fundus may easily be obtained, no bubbles were ever seen even in animals with severe dyspnoea, so that the method cannot be taken as giving any indication of the absence of bubbles in the blood. Some of these animals died and plenty of free gas was found in the retinal vessels post-mortem. Four animals which showed no symptoms were killed within ten minutes after decompression with the following results:

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[1] The vessels and bladder in the rabbit are so thin-walled that bubbles can be seen with certainty if they are present.

[2] On the other hand dogs (showing no symptoms) killed after sudden decompression after 16 minutes exposure at 2·8 atmospheres, 5+ at 3·5, 12 at 4·5 and 5 at 4·7, showed no bubbles in the blood: these were however found in three other dogs after 10+ minutes at 4·0 atmospheres, 16 and 72 at 4·5.

TABLE XXIV.

Series and number of goat	Pressure lbs.	Exposure (actual) minutes	Decompression minutes	Bubbles in blood	Results of similar experiments in other goats			
					Number	Bends	Severe symptoms	Death
3 : XXVI A	25	120	½	absent	23	2	0	0
1 : II	45	26	6	absent	15	3	0	0
2 : XIV	75	19	31 stages	present	29	5	0	0
1 : 1	78	30	9	absent	4	0	0	1

These goats were killed before the expiration of the appropriate period for the development of bends. The experience of similar experiments indicates that they might have shown symptoms if they had not been killed. Yet three out of four had no bubbles in the blood. A few observations on rabbits on the other hand gave rather different indications. Seven rabbits in seven different experiments were exposed to 75 lbs. for periods of from 15 to 120 minutes and killed immediately after decompression in 31 minutes by stages. There is no question but that it would be the very rarest occurrence for a rabbit to have any symptoms under these circumstances, but in four of the animals we found bubbles in the heart or great veins. These may however have been formed post-mortem, and in one such case they were observed to appear some time after death.

The post-mortem appearances observed may now be shortly described. It should be remembered that most of the animals dealt with here died under circumstances of experiment less severe than those of other observers. The pressure was in almost all cases 75 lbs. and in a majority of instances the decompression was not instantaneous. These considerations probably afford the explanation of the somewhat less emphatic changes which we have noted. The naked eye appearances were in nearly every case supplemented by microscopical examination of many of the organs.

Lungs. The amount of blood in the lungs depends upon the condition of the heart: in severe cases, with the pulmonary artery choked with bubbles and the right heart distended, they are pale and bloodless; in other instances the quantity of blood appears about normal.

Haemorrhages may occur and small blood clots are not infrequently found in the trachea. A very marked, scattered, lobular emphysema is almost constant; the only explanation appears to be that some bronchioles are more or less impervious during decompression. Examination of fresh material showed that bubbles may burst out of the capillaries into the interstitial tissue of the lungs, and presumably therefore also into the alveoli. The same was found in rabbits which were killed by the injection of small quantities of air into the veins. Nothing resembling the exudative process seen in oxygen poisoning was ever found in animals exposed to simple air pressures.

In all fatal cases (with one exception) more or less abundant bubbles were found in the blood. In severe, rapidly fatal cases, the right heart is much distended with bubbles, the pulmonary vessels plugged with froth and the left heart nearly empty. The block in the pulmonary artery may indeed be so complete that the left auricle is collapsed and puckered up. In other animals, which have lived for 20, 30 or 60 minutes or longer, the two sides of the heart are equally full of blood and the right heart is not distended. The immediate cause of death, in all but three cases, which died many hours after decompression, was clearly pulmonary air embolism, and this is doubtless the cause of the urgent dyspnoea already noted. In two cases death ensued without any dyspnoea. Both these animals were intentionally killed by very severe experiments, viz. 75 lbs. for 1 hour (goat XXI) and 3 hours (XVIII A) with decompression in $1\frac{3}{4}$ and 1 minutes. Both showed no symptoms for 5 minutes and collapsed and died quietly in 10 and 12 minutes respectively. Such animals must be regarded as being so overwhelmed by sudden asphyxiation that they exhibit only the symptoms of deficiency of oxygen and not those of the accumulation of carbon dioxide.

The fatal case in which no bubbles were found in the blood post-mortem was in goat XXVII A. After 75 lbs., 15 minutes, 42 seconds it showed pain, ate part of a note-book, and became paraplegic. It was found dead next morning[1]. In several other cases of delayed death, and in one (XVI A) in which the animal died in 3 hours[2], the quantity of bubbles found seemed to be altogether inadequate to produce a fatal result. One may suppose that they had been previously more numerous and that oxygen starvation resulted in death at a time when the aeration of the blood had been restored. This form of delayed death from deficiency of oxygen is well-known in *e.g.* carbon monoxide poisoning. It might be for instance that temporary obstruction of the coronary arteries or portal capillaries caused fatal degenerative changes in the heart muscle or liver cells.

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[1] Heller, Mager and v. Schrötter (p. 852) record a case in a dog fatal in 6 hours after decompression in which no free gas was found in the vessels.

[2] This animal had however been recompressed and died under a state of recompression: see protocol *e*, Appendix III.

The distribution of the bubbles in the different parts of the vascular system shows several peculiarities. If only a few are present, all may be collected in the smaller branches of the pulmonary artery with none in the right heart. The left heart generally contains a few bubbles; the amount there and in the arteries roughly varies inversely with the rapidity of death unless decompression has been very quick. Smallness in the amount of bubbling affords the heart the best chance of being able to pass on the froth to the arteries, and cases which die slowly seem to show distinctly more arterial bubbles than those which expire almost at once. The veins contain variable quantities of bubbles, but always more than the arteries. They are especially abundant in the mammary, mesenteric, spermatic and portal veins, coronary vessels, and notably few in the veins on the surface of the stomach and in those of the brain and spinal cord. In several instances we have noticed great accumulations of froth in the liver while the spleen at the same time showed no bubbles in the blood flowing out on section. This massive portal embolism is probably the cause of the multiple small capillary haemorrhages which are frequently seen in the omentum and mesentery. Blocking of the portal circulation might also give rise to general symptoms of a very serious character.

It should be noted that the liver is particularly badly situated for getting rid of excess gas during and after decompression; nearly all the blood reaching it is already partly saturated by passing through the intestines, &c. The liver also contains much fat.

Lymph. The lymph in the thoracic duct has been noted to be full of froth on several occasions.

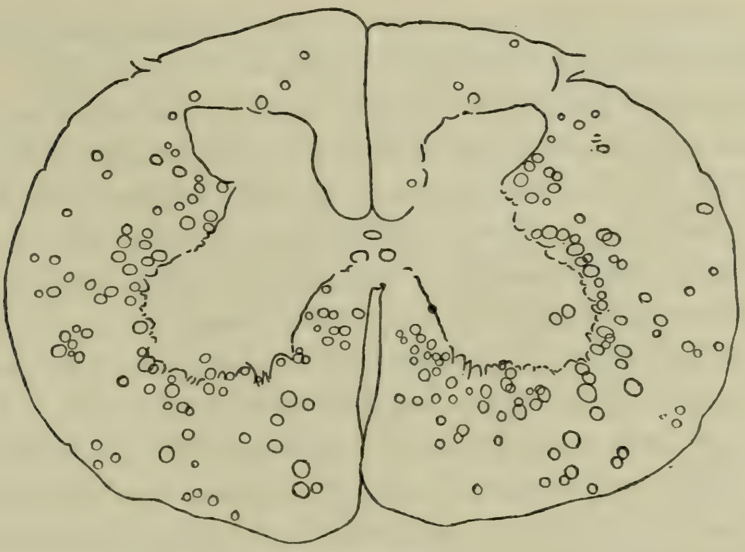
Other liquid areas. Bubbles have very seldom been found in the *aqueous humour*; the blood supply is considerable, so that their absence is probably to be attributed to the excess gas being carried off during decompression and the period which the supersaturation phenomenon adds, for practical purposes, to the actual time occupied in reducing the pressure to normal. The *vitreous humour*, on the other hand, has a poor blood supply and its consistence is such that any bubbles forming there would remain *in situ*. On only one occasion have bubbles been found (goat XXVI, 75 lbs., 2 hours, 31 minutes stages), when they were seen in a layer close against the ciliary body. Their absence is explicable on the ground that the vitreous humour would take a very long time to saturate. The *bile* often contains bubbles: they were noted in one goat exposed at 75 lbs. for 15 minutes and killed immediately after decompression in 31 minutes stages, and in 8 animals exposed to the same pressure for 1—4 hours, but not in two animals exposed for 15 minutes which died 30 minutes after decompression in 10 minutes and 30 minutes uniformly respectively. The *urine* found in the bladder post-mortem is remarkably free from bubbles; on two occasions only has free gas been found. We have evidence here that the phenomenon must be due to supersaturation and the absence of "points," since we have very frequently observed goats pass urine after decompression which frothed freely on coming into contact with foreign surfaces. This is often seen in animals which show no symptoms. Thus in one experiment, seven goats were exposed at 45 lbs. for one hour and decompressed in 30 seconds. One had bends 19 minutes later. Within 24 minutes after decompression four animals passed urine; in two cases this frothed up freely as it ran over the pavement while in the other two (including the goat which had bends) no bubbles could be observed. It is somewhat striking to observe the transparent bladder of the rabbit containing urine quite free from bubbles while the vesical veins coursing over its surface are full of froth. The *cerebro-spinal fluid* rarely shows any bubbles: they have been seen only three times, in all cases after long exposures (1 to 3 hours) at 75 and 51 lbs. with sudden decompression. *Synovial fluid* is almost always full of bubbles; exposure for 15 minutes at 75 lbs. is sufficient to cause their presence, while decompression in 100 minutes uniformly is not enough to prevent their formation. In animals which have died within 3 hours of decompression, we have found them in every case. *Amniotic fluid* is dealt with below. Bubbles have been seen after very severe experiments in the *pericardial* and *peritoneal fluids* when present, and in the serous contents of a mammary cyst, but not in the milk. We have not seen any accumulations of gas in the serous cavities.

Solid organs. Fat commonly shows bubbles, often in extreme abundance. They are more numerous in the abdominal than in the subcutaneous fat; the latter is much more vascular. Other solid organs for the most part show no bubbles outside the blood vessels; a few are sometimes found in the liver and the spinal cord may contain large numbers. In the liver it is very difficult to determine whether any bubble is inside a capillary or not, and we have failed to find clear evidence of bubbles outside blood-vessels, still less actually within tissue cells, in cardiac or skeletal muscle, spleen, kidney, suprarenal, salivary glands, thymus, thyroid and parathyroid, pancreas, lymphatic glands, haemolymph glands, nerves, posterior root ganglia, testis, ovary or mammary gland, though an extensive systematic histological examination has been made of more than 20 goats exposed at 75 or 45 lbs. for from 10 minutes to 4 hours and decompressed in from 30 seconds to 100 minutes.

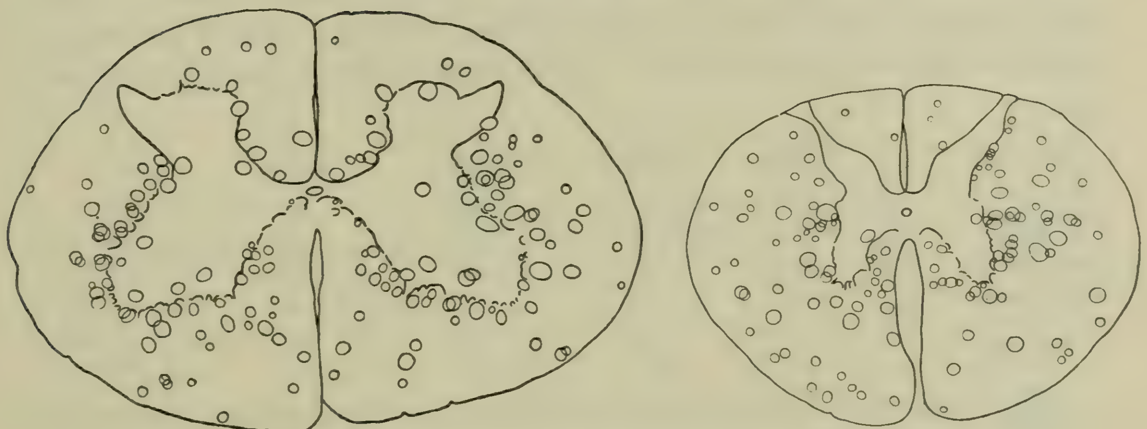
This condition has no very obvious explanation. It is curious, for instance, to see the spermatic vein (and sometimes artery as well) full of froth and yet find no evidence of bubbles in the tissues which it drains: the same thing is also shown most strikingly in the mammary gland and vessels. There can be no doubt that these tissues must be fairly completely saturated in 4 hours and it is impossible that the excess should be removed from the tissues more quickly than from the blood. It follows that the blood must stand in an unfortunate relation towards bubbling in that it effervesces with a smaller difference of pressure, within and without, or with the same difference of pressure in a shorter time, than do the more solid organs. It seems unlikely that this difference depends on the motion of the blood. Rhythmical pulsating circulation through a smooth elastic system can hardly function as a shaking which would be efficient in bringing out free gas. Even if it did, it is not easy to see why the tissues are not affected by the pulsations in the same way, though perhaps not to the same degree, since isolated collections of fluid, as in the joints, may bubble very easily. One can only suppose that the dissolved particles of gas find in the tissues obstacles, visible and invisible, more obstructive to their aggregation into bubbles than those occurring in the blood.

Bubbles once formed in the blood will also increase in size more readily, since their movement will continuously keep them in contact with fresh portions of supersaturated liquid.

Among the solid organs, bubbles outside the vessels are found most frequently in the central nervous system. The fatty nature of this tissue is probably important in this respect. The brain is singularly free, both by direct examination and by the study of secondary degenerations. The cord may however contain numerous bubbles, and a study of their occurrence and distribution gives interesting results. In the first place they may occur in areas of softening after comparatively mild experiments (*e.g.* 45 lbs., 2 hours, 10 minutes): in this case

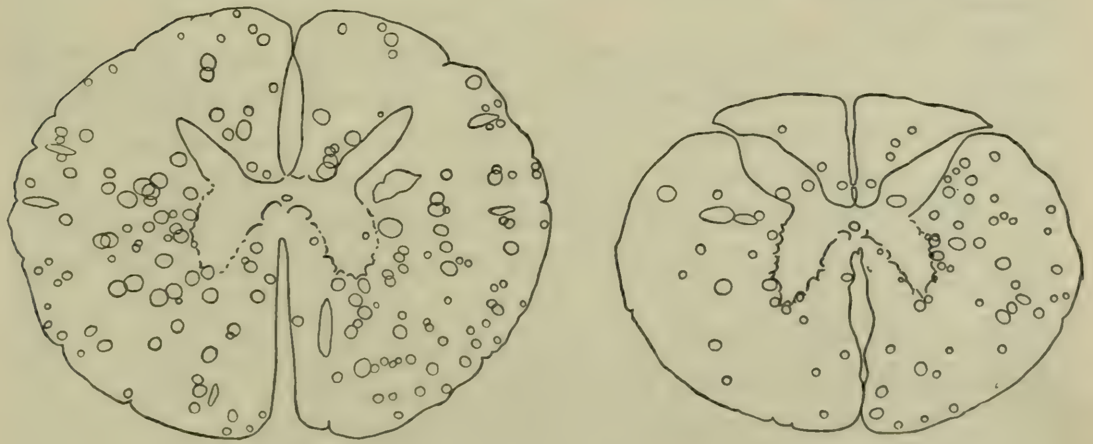


6th cervical.



4th lumbar.

11th dorsal.



2nd cervical.

3rd dorsal.

Fig. 7. Shows the distribution of extravascular bubbles in five regions of the spinal cord of goat 3 (series IV). The animal died of oxygen poisoning soon after the beginning of a decompression of 133 minutes duration by stages after 3 hours exposure at 81 lbs. in an atmosphere containing 36 % oxygen. The bubbles are practically confined to the white matter and are there especially concentrated in the boundary zone where the circulation is least good. Each diagram is a composite drawing showing all the bubbles in 0·4 mm. length of cord.

they are confined to the area in which the circulation has been brought to a sudden standstill by a collection of gas. On the other hand they may be found in the cords of animals which have died immediately after a drastic decompression. This is however rather exceptional. Thus the cords of three animals decompressed in less than a minute after 1 hour at 100 lbs. and 1 and 2 hours at 75 lbs. contained numerous bubbles, while in two animals treated in the same way after exposure at 75 lbs. for 1 and 3 hours respectively, none were found.

The distribution of the bubbles when numerous is in harmony with the theoretical conclusions derived from the blood supply. They are for instance least numerous in those segments with an abundant blood supply (lumbar enlargement), and are almost confined to the white matter, those found in the grey substance being distributed along its periphery towards the boundary zone between the superficial and deep vessels. Thus one cord (goat XXI: 75 lbs., 1 hour, $1\frac{3}{4}$ minutes) contained the following bubbles in 412 cubic millimetres in different parts:

Segment	Grey matter	Posterior columns	Ant.-lat. columns	White matter	Total
2nd cervical	14	141	215	197	175
5th dorsal	11	23	95	87	79
1st lumbar	0	32	161	140	140
4th lumbar	2	15	37	28	19
Average	7	53	127	113	103

Fig. 7 shows the distribution of bubbles in another case: note the paucity of bubbles in the grey matter and their concentration in the boundary zone.

The distribution of the areas of softening is also important. With one exception, these are most marked in, and usually confined to, the lower dorsal and upper lumbar segments where the blood supply of the cord may on many grounds be surmised to be at its minimum. They affect only the white matter. Now the only parts of the body in which we have found appearances resembling embolic infarction are the white matter of the spinal cord and the fat. The latter has on several occasions been found to contain large and small areas of necrosis. We have obtained no evidence of infarction of the spleen, kidney, heart-muscle, &c. The distribution of small bubbles by the arterial blood stream must be universal. They probably lodge in many places: while they are rapidly pushed forward in the grey matter and in most other tissues, if they lodge among the fatty surroundings of the capillaries of the white matter, or in actual fat, they quickly increase in size to such an extent that their removal becomes impossible. It is also clear that in consequence of the slow circulation in the white matter, and especially in such inactive parts as the lower dorsal segments, bubbles have plenty of time to increase in size in the circulating blood. The condition of supersaturation will also last much longer in the white than in the grey matter. The cause then of these areas of softening in the cord is not ordinary embolism, but embolism which becomes effective to produce infarction by reason of the effect on the size of the embolus of the local conditions of the circulation rather than from any of those peculiarities in the resistance of the different tissues to lack of oxygen, or in the freedom of collateral circulation, which determine the topography of common infarcts.

The presence of bubbles in the uterine contents. We may group together here a number of casual observations which have been made on the distribution of bubbles in the foetus and amniotic fluid of pregnant goats dying of caisson disease. The pressure was in all cases 75 lbs.

TABLE XXV.

Number of goat	Exposure minutes	Decompression minutes	Time of death after decompression minutes	Bubbles present in the			Development of foetus
				maternal blood	foetus	amniotic fluid	
XXIII	15	31 stages	30	+	0	0	advanced
XXII	120	31 ,,	75	+	0	+	,,
XX	240	31 ,,	40	+	live +	+	6 inch
					dead 0	+	4 inch
XXI	60	1¾	10	+	+	+	advanced
XI	240	31 uniform	25	+	0	0	1 inch
XIV	15	31 stages	killed at once	+	0	0	½ inch
XVIII A	180	1	12	+	+	+	advanced
XXVIII A	180	4¼	24	+	0	0	½ inch
XXXII A	180	4	27	+	0	0	4 inch (dead)

These observations seem to be fairly concordant. In 15 minutes the uterine contents have not taken up much excess of gas (XXIII), nor does a dead foetus absorb any (XX, XXXII A). In one hour both foetus and amniotic fluid have taken up abundant excess (XXI), which may, if death be long delayed after a rather slow decompression, be discharged from the foetus more quickly than from the amniotic fluid (XXII)[1]. With a very young foetus, the circulation is probably too active and the bulk of fluid too small to favour bubbling (XI, XIV, XXVIII A).

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[1] Two pregnant guinea-pigs were exposed for 1 hour at + 100 lbs. and decompressed in 34 minutes by stages: they showed no symptoms. On being killed 5 hours later, numerous bubbles were found in the amniotic fluid but nowhere else.

The amniotic fluid, which contained in this case only a faint trace of proteid, may show the phenomenon of supersaturation to an exquisite degree. In goat XXI it was especially noted that a large bubble was present in the amniotic fluid on removing the uterus from the body. After free shaking to bring out any more gas, the uterus was opened and the contents poured into a glass vessel. Contact with this foreign surface immediately produced a great froth of fine bubbles.

The free gas runs together into one large bubble. Advantage was taken of this convenient circumstance in two instances to make analyses. The samples were collected over water and in XXII analysed at once; in XX they were kept for 20 hours over water before examination and in this case therefore the figures for CO_2 represent minimal and those for oxygen maximal values.

	XXII	XX	XX
		Live foetus	Dead foetus
Total gas c.c.	16	27	10·5
CO_2 per cent.	16·23	3·55	2·73
O_2 ,,	1·10	2·14	0·85
N_2 ,,	81·90	94·14	96·21
Combustible gas (calculated as CH_4 and H_2)	0·77	0·17	0·21

These results correspond with those of Bert (pp. 955, 961) of the free gas in the heart: they are not in accord with those of Heller, Mager and von Schrötter (p. 800) who found 15·31 and 7·18 per cent. of oxygen in the free gas collected from the hearts of dogs. It is somewhat significant that if this excess of oxygen is calculated as an air leak, the figures of Schrötter correspond exactly with those of Bert and our analysis XXII.

Duration of bubbles. It is difficult to say how long bubbles may remain in the vessels and tissues after their first formation in animals which survive[1]. The question is much complicated by the fact that we have reason to believe that bubbles may continue to form for a long, and quite unknown, time after decompression. This is probably especially marked in cases in which either local blocking of the blood supply has occurred, or the circulation has been slowed generally by a greater or less degree of cardiac and pulmonary obstruction. It would appear likely that bubbles once lodged in the lungs would probably stop there for a considerable time, since their gaseous composition would quickly approximate to that of the alveolar air and there would be no considerable difference of tension to encourage their removal. Such results as we have which bear on the matter are collected in the next table. It will be seen that bubbles have been found in the blood of one animal which died two days after decompression (and that in an animal which had shown no dyspnoea) and in the joints up to 26 hours. In the substance of the spinal cord bubbles may persist far longer: in two cases we have found them 15 days after the last exposure to pressure and in one 27 days after the last occurrence of symptoms.

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[1] Zografidi (*Revue de Médecine*, 1907, p. 159) records the finding of numerous bubbles in the peripheral vessels, but not in the heart, of a diver who was paralysed and died 33 days after decompression !

TABLE XXVI.

Goat	Pressure lbs.	Exposure minutes	Decompression minutes	Result hours	Bubbles present in	
					Blood	Joints
XXII (Series I)	75	35	40 stages	died 39	+	–
XXV A	75	120	100 uniform	,, $16\frac{1}{2}$	+	0
XXVII A	75	15	$\frac{4}{6}$	,, 15*	0	0
XV A	45	120	$\frac{1}{2}$	killed 24	0	0
4	45	120	$\frac{1}{2}$	,, 26	0	+
XIII A	45	120	10 uniform	,, 72	0	0
X A	75	10	$\frac{5}{6}$	,, 96	0	0
XXIII A	45 to -6	180	6	,, 144	0	0

* Found dead next morning.

A histological point of some practical importance arises in connection with the size of the bubbles found in the blood. The bubbles soon run together into large bullae after death so that it is necessary to make the examinations immediately after death in order to observe approximately the true state of affairs. It will then be found that there are no bubbles so small as to be of strictly microscopic dimensions. Nor are any very small bubbles found in the spinal cord; in any one case all the bubbles are about the same diameter, commonly some 25 microns. The same is true of the bubbles given off on decompressing water, salt solutions, serum, blood, and even such thick solutions as gelatine or agar. At the same time however it is possible to produce bubbles which are truly microscopic and which last some hours in some sticky solutions such as gum and treacle. The energy required to aggregate particles of dissolved gas into a bubble is evidently considerable, and there is the same difficulty in the formation of free gas bubbles from solution in liquids as there is in the separation of liquid particles from solution in gases and of solid particles from solution in liquids.

Extremely minute bubbles are unstable in the same way as extremely minute droplets of water condensing from supersaturated air, or salt crystals forming in a supersaturated solution in water: in all cases the tendency is to reduce the free energy (surface tension) by reducing the ratio of surface to mass, and accordingly the smallest bubbles, droplets or crystals as the case may be, are rapidly, in the case of bubbles practically instantaneously, abolished to produce macroscopic masses. This is well seen on watching under the microscope a stream of bubbles coming off some "point" in soda water. It follows that if the concentration of dissolved molecules of gas is not higher than some unknown point, bubbles will not be formed. It is possible that the absence of bubbles from most of the solid tissues is to be explained by this non-existence of very small bubbles and the mechanical difficulties of the rapid aggregation of a sufficient number of molecules to produce large bubbles. It is also doubtless connected with the period of delay in bubble formation whereby an animal, for practical purposes, gains several minutes over the actual time of decompression.

It is reasonable to suppose that the temporary paralyses are due to temporary ischaemia from air bubbles in the vessels. The more lasting palsies are undoubtedly caused by obstruction sufficiently complete to produce softening and necrosis. As already mentioned, the change is confined to the white matter and in nearly all instances affects only the lower dorsal or upper lumbar region. In these segments the bulk of cord destroyed may be very extensive: thus in goat XXIII A fully three-quarters of the lateral columns were destroyed from the eighth dorsal to the second lumbar segment over a length of rather more than five inches, and in goat XV A the softening involved nearly the whole of the lateral columns and parts of the anterior and posterior columns for a length of four and a half inches. Such cases may recover to a remarkable degree, and eventually show objective signs of paralysis so slight as to be hardly perceptible except to those familiar with the individual animals.

The only other tissue in which we have found any signs of the results of circulatory obstructions is the intra-abdominal fat. Large masses of necrosed fat have been occasionally met with, especially in the fat lying below the kidneys. Only late stages have been seen: the necrosed areas are then surrounded by a well-marked ring of giant-cell reaction, and the surface layers are mostly converted into a calcium soap.

No evidence of infarction in other organs has been seen: the rarity of such changes seems to afford pretty good evidence that the duration of any obstruction in such organs as the spleen or kidney cannot be very long.

The pathological changes underlying the chief symptoms have been already sufficiently noticed except as regards bends. The exact cause of bends is not known. They have been attributed to bubbling in the central nervous system, chiefly on the ground that human experience shows that they are very frequently bilaterally symmetrical. This fact however cannot be taken as indicating any such origin in view of the complete symmetry of the limbs (where the symptoms occur) and the uniform symmetry of the causative agent throughout the body. In two animals which were killed soon after decompression when they showed bends only, we could find nothing abnormal in the cord, posterior root ganglia, or nerves, and there is abundant evidence from a number of goats that the cause of bends does not produce such lesions in the nervous system as are followed by secondary degeneration which can be revealed by the methods of Marchi or Weigert. The two following goats may be cited in detail as to this point: in neither was any degeneration found. Goat XXI (Series II) was used in seven experiments between November 26th and January 18th: it had bends on December 5th, 11th and 18th (the last being noted as "bad bends"), and dyspnoea, nearly fatal, on January 2nd: it was killed on January 18th. Goat XV A (Series III) was exposed 27 times between February 2nd and June 10th: it had bends on February 2nd, 20th, 22nd, March 3rd, 5th, May 13th, 15th, 27th and June 6th: it was killed on June 11th. Thorough examination of the pons, medulla and cord showed no secondary degeneration in either animal. There are therefore reasons for thinking that the cause of bends is peripheral rather than central. The constant presence of bubbles in the joints has been already mentioned, and they seem to afford a fairly probable explanation of most of the cases. Even in those cases in which the muscles are the seat of pain, it is quite possible that a sensation actually originating in or around the joints is referred to other parts. The joint pains in man are often relieved by flexion, and goats evidently try to obtain ease in the same way (see Plate VI). This fact adds strong confirmation to the conclusion that the origin of the pain is in or about the joints.

We have already seen that bends, while not the first symptom to appear as the duration of exposure to high pressure is increased, are the last symptom to disappear as the decompression is extended, that bends in short arise in parts of the body which saturate and desaturate rather slowly. The synovial fluid satisfies this criterion; on the other hand the tendons and other dense tissues about the joints are not in disagreement with it.

Bends occur with a lower degree of supersaturation with air than any other symptom of compressed-air illness. In goats they are readily produced after exposures to 30 lbs. or less. The fact that only a moderate degree of supersaturation is needed to produce them seems to explain the fact that although they are not the first symptom to appear as the duration of exposure to very high pressure is increased, yet a moderate duration of exposure suffices to produce them, in spite of the fact that they occur in parts of the body with a slow rate of circulation, as shown by the fact that they are the last symptom to disappear as the duration of decompression is prolonged.

SUMMARY.

1. The time in which an animal or man exposed to compressed air becomes saturated with nitrogen varies in different parts of the body from a few minutes to several hours. The progress of saturation follows in general the line of a logarithmic curve and is approximately complete in about five hours in man and in a goat in about three hours.

2. The curve of desaturation after decompression is the same as that of saturation, provided no bubbles have formed.

3. Those parts of the body which saturate and desaturate slowly are of great importance in reference to the production of symptoms after decompression.

4. No symptoms are produced by rapid decompression from an excess pressure of 15 pounds, or a little more, to atmospheric pressure, *i.e.* from two atmospheres absolute to one. In the same way it is safe to quickly reduce the absolute pressure to one-half in any part of the pressure scale up to at least about seven atmospheres: *e.g.* from six atmospheres (75 pounds in excess) to three (30 pounds), or from four atmospheres to two.

5. Decompression is not safe if the pressure of nitrogen inside the body becomes much more than twice that of the atmospheric nitrogen.

6. In decompressing men or animals from high pressures the first part should consist in rapidly halving the absolute pressure: subsequently the rate of decompression must become slower and slower, so that the nitrogen pressure in no part of the body ever becomes mor

than about twice that of the air. A safe rate of decompression can be calculated with considerable accuracy.

7. Uniform decompression has to be extremely slow to attain the same results. It fails because it increases the duration of exposure to high pressure (a great disadvantage in diving work), and makes no use of the possibility of using a considerable difference in the partial pressure of nitrogen within and without the body to hasten the desaturation of the tissues. It is needlessly slow at the beginning and usually dangerously quick near the end.

8. Decompression of men fully saturated at very high pressures must in any case be of very long duration: and to avoid these long decompressions the time of exposure to such pressures must be strictly limited. Tables are given indicating the appropriate mode and duration of decompression after various periods of exposure at pressures up to 90 pounds in excess of atmospheric pressure.

9. Numerous experiments on goats and men are detailed in proof of these principles.

10. The susceptibility of different animals to compressed-air illness increases in general with their size owing to the corresponding diminution in their rates of circulation.

11. The average respiratory exchange of goats is about two-thirds more than that of man; they produce about 0·8 gram. of CO_2 per hour per kilogramme of body weight.

12. The mass of the blood in goats is six and a half or seven and a half per cent. of the "clean" body weight.

13. The individual variation among goats in their susceptibility to caisson disease is very large. There is no evidence that this depends directly on sex, size or blood-volume: there is some evidence that fatness and activity of respiratory exchange are important factors.

14. Death is nearly always due to pulmonary air-embolism, and paralysis to blockage of vessels in the spinal cord by air. The cause of "bends" remains undetermined; there are reasons for supposing that in at least many cases they are due to bubbles in the synovial fluid of the joints.

15. In our experiments bubbles were found post-mortem most freely in the blood, fat and synovial fluid; they were not uncommon in the substance of the spinal cord, but otherwise were very rarely found in the solid tissues.

APPENDIX I.

Details of the experiments made on Lieutenant Damant and Mr A. Y. Catto, Gunner, R.N., in the pressure chamber at the Lister Institute.

These experiments were undertaken in July, 1906, as a preliminary to actual diving experiments in very deep water.

In the first three or four the decompression was controlled from inside the chamber; in the rest from outside. The subjects remained closed in the chamber for half an hour after each experiment, the engine being also kept running so that recompression could be at once begun if any serious symptom developed. In addition to the actual period of exposure to each pressure, we have noted the virtual period of exposure calculated on the assumption that about half the time occupied in compression must be added (see above, p. 362).

In view of the results with goats, the occurrence of decompression symptoms seemed probable in the more severe experiments. No symptoms were, however, observed, except considerable itching of the skin of the fore-arms where it was uncovered. In the compressed air the well-known alteration in the voice, and corresponding abnormal sensations about the lips and mouth, were very marked at pressures exceeding 60 or 70 lbs.

I. July 25th. Actual exposure to 39 lbs. for one hour. Virtual exposure 69 minutes, decompression in 24 minutes:

Compressed to	39 lbs.	in 17 minutes.	
Waited at	39 ,,	for 60 ,,	
Decompressed to	9 ,,	in 7 ,,	24.
Waited at	9 ,,	for 5 ,,	
Decompressed to	4 ,,	in 1 ,,	
Waited at	4 ,,	for 9 ,,	
Decompressed to	0 ,,	in 2 ,,	

II. July 26th. Actual exposure to 50 lbs., 27 minutes. Virtual exposure, 39 minutes. Started at 10.37 a.m. Decompression in 34 minutes:

Compressed to	50 lbs.	in 24 minutes.
Waited at	50 ,,	for 27 ,,
Decompressed to	17 ,,	in 4 ,,
Waited at	17 ,,	for 6 ,,
Decompressed to	13 ,,	in 1½ ,,
Waited at	13 ,,	for 3½ ,,
Decompressed to	9 ,,	in 2 ,,
Waited at	9 ,,	for 3 ,,
Decompressed to	4 ,,	in 2 ,,
Waited at	4 ,,	for 8 ,,
Decompressed to	0 ,,	in 4 ,,

III. Same day, 3.3 p.m. Exposure to 55 lbs. for 19 minutes. Virtual 33 minutes. Decompression in 31 minutes:

Compressed to	55 lbs.	in	28 minutes.
Waited at	55 ,,	for	19 ,,
Decompressed to	17 ,,	in	4 ,,
Waited at	17 ,,	for	5 ,,
,, ,,	13 ,,	,,	5 ,,
,, ,,	9 ,,	,,	5 ,,
,, ,,	4 ,,	,,	10 ,,
Decompressed from	4 to 0 ,,	in	2 ,,

The time taken for decompressing from 17 to 13 lbs., &c., was counted as time at 13 lbs.

IV. July 27th, 10.29 a.m. Exposure to 60 lbs. for 20 minutes. Virtual exposure 36 minutes. Decompression in 37½ minutes:

Compressed to	60 lbs.	in	30½ minutes.
Waited at	60 ,,	for	20 ,,
Decompressed to	22 ,,	in	5 ,,
Waited at	22 ,,	for	5 ,,
Decompressed to	17 ,,	in	1 ,,
Waited at	17 ,,	for	4 ,,
Decompressed to	13 ,,	in	1½ ,,
Waited at	13 ,,	for	3½ ,,
Decompressed to	9 ,,	in	1 ,,
Waited at	9 ,,	for	4 ,,
Decompressed to	4 ,,	in	1½ ,,
Waited at	4 ,,	for	8½ ,,
Decompressed to	0 ,,	in	2½ ,,

V. Same day, 3.37 p.m. Exposure to 67 lbs. for 18 minutes. Virtual exposure 36 minutes. Decompression in 36 minutes:

Compressed to	67 lbs.	in	36 minutes.
Waited at	67 ,,	for	18 ,,
Decompressed to	22 ,,	in	3 ,,
Waited at	22 ,,	for	5 ,,
Decompressed to	17 ,,	in	1 ,,
Waited at	17 ,,	for	4 ,,
Decompressed to	13 ,,	in	1 ,,
Waited at	13 ,,	for	4 ,,
Decompressed to	9 ,,	in	1 ,,
Waited at	9 ,,	for	4 ,,
Decompressed to	4 ,,	in	1½ ,,
Waited at	4 ,,	for	8½ ,,
Decompressed to	0 ,,	in	3 ,,

VI. July 30th, 10.57 a.m. Actual exposure at 74 lbs., 15 minutes. Virtual exposure 35 minutes. Decompression in 42 minutes:

Compressed to	74	lbs.	in	39	minutes.
Waited at	74	,,	for	15	,,
Decompressed to	26	,,	in	4	,,
Waited at	26	,,	for	5	,,
Decompressed to	22	,,	in	1	,,
Waited at	22	,,	for	4	,,
Decompressed to	17	,,	in	1½	,,
Waited at	17	,,	for	3½	,,
Decompressed to	13	,,	in	1	,,
Waited at	13	,,	for	4	,,
Decompressed to	9	,,	in	1	,,
Waited at	9	,,	for	4	,,
Decompressed to	4	,,	in	1½	,,
Waited at	4	,,	for	8½	,,
Decompressed to	0	,,	in	3	,,

VII. July 31st, 11.0 a.m. Actual exposure to 80 lbs. for 12 minutes. Virtual exposure, 34 minutes. Decompression in 51 minutes:

Compressed to	80	lbs.	in	44	minutes.
Waited at	80	,,	for	12	,,
Decompressed to	31	,,	in	3	,,
Waited at	31	,,	for	5	,,
Decompressed to	22	,,	in	1	,,
Waited at	22	,,	for	4	,,
Decompressed to	18	,,	in	1	,,
Waited at	18	,,	for	4	,,
Decompressed to	15	,,	in	3	,,
Waited at	15	,,	for	2	,,
Decompressed to	13	,,	in	1	,,
Waited at	13	,,	for	4	,,
Decompressed to	9	,,	in	1	,,
Waited at	9	,,	for	9	,,
Decompressed to	4	,,	in	2	,,
Waited at	4	,,	for	8	,,
Decompressed to	0	,,	in	3	,,

APPENDIX II[1].

A DIARY OF THE DEEP DIVING EXPERIMENTS CARRIED OUT OFF ROTHESAY, ISLE OF BUTE, FROM H.M.S. *SPANKER*, AUGUST, 1906.

Monday, 20th August.

H.M.S. *Spanker* arrived at Rothesay about 7 p.m., and was met by Drs Haldane and Rees and Mr Catto, Gunner, R.N. Arrangements were made to commence experiments the following day.

Tuesday, 21st August.

All the pumps to be used in the experiments were tested up to a pressure of 200 feet, and the leakage at this pressure measured. The pressure gauges, which had been specially graduated for these experiments, were tested and found to give correct readings. The method of testing employed was to attach the free end of the diving hose to a lead line, and lower it over the side into the sea to the required depth. The pumps were then hove round until there was a free supply of air, and then stopped whilst the reading of the gauge was taken.

The re-compression chamber was tested on the Whitehead torpedo charging column, and it was found that the pressure could be brought up to 40 lbs. on the gauge in 3 minutes. There was a leak of 1 lb. per minute, or, roughly 3 cubic feet. Afterwards Drs Haldane and Rees were compressed up to about 30 lbs. in order to further test the working of the chamber.

In the afternoon both divers made a trial dive in 15 fathoms:

	Lieutenant Damant	Mr Catto
Time of descent	2 minutes	1¼ minutes.
,, on bottom	1 hour	1 hour.
,, of ascent	18½ minutes	17½ minutes.
No. 5-minute stops	1 at 30 feet	1 at 20 feet.
,, 10 ,, ,,	1 ,, 10 ,,	1 ,, 10 ,,

Two double pumps were used for each diver in these and the subsequent dives. The divers were perfectly comfortable in moving about on the bottom. It may be mentioned that Lieutenant Damant had not dived previously beyond about 19 fathoms, and had no experience in diving except what he had gained in his course of instruction as a gunnery officer and in experimenting at Portsmouth for the Committee. Mr Catto had much previous experience in diving work, but had never dived beyond 23 fathoms.

[1] Reprinted from the *Report of the Admiralty Committee on Deep Diving*, 1907.

Wednesday, 22nd August,

H.M.S. *Spanker*, off Rothesay.

In the forenoon Mr Catto descended in 23 fathoms, and in the afternoon Lieutenant Damant did the same:

	Mr Catto	Lieutenant Damant
Time of descent	2 minutes	2½ minutes.
,, on the bottom	20 ,,	20 ,,
,, of ascent	35½ ,,	32½ ,,
No. 5-minute stops	4 at 50, 40, 30, 20 feet	2 at 50, 40 feet.
,, 10 ,, ,,	1 ,, 10 feet	2 ,, 20, 10 ,,

Thursday, 23rd August,

H.M.S. *Spanker*, off Rothesay.

After testing the pumps each diver made a descent to 25 fathoms:

	Lieutenant Damant	Mr Catto
Time of descent	2 minutes	2 minutes.
,, on the bottom	18¾ ,,	19¼ ,,
,, of ascent	37¾ ,,	39¾ ,,
No. 5-minute stops	3 at 60, 45, 30 feet	3 at 50, 40, 30 feet.
,, 10 ,, ,,	2 ,, 20, 10 feet	2 ,, 20, 10 feet.

Friday, 24th August,

H.M.S. *Spanker* was taken through the narrows of the Kyles of Bute and anchored off the entrance of Loch Riddon.

In the morning, after the usual tests had been applied to the pumps, Mr Catto descended in 27 fathoms, and in the afternoon Lieutenant Damant went down in a similar depth:

	Mr Catto	Lieutenant Damant
Time of descent	2 minutes	1 minute 20 seconds.
,, on the bottom	16½ ,,	16¾ minutes.
,, of ascent	55½ ,,	44¼ ,,
No. 5-minute stops	4 at 60, 50, 40, 30 feet	4 at 60, 50, 40, 30 feet.
,, 10 ,, ,,	1 at 20 feet. (Diver was employed just under the ship's bottom in examining a propeller which had been slightly injured, for 19½ minutes before coming up.)	2 ,, 20, 10 feet.

Saturday, 25th August,

H.M.S. *Spanker*, off Loch Riddon.

The *Spanker* shifted her position slightly, and, after the usual tests of the pumps, both divers descended in 29 fathoms of water:

	Mr Catto	Lieutenant Damant
Time of descent	3 minutes	1½ minutes.
,, on the bottom	14½ ,,	13½ ,,
,, of ascent	46 ,,	48½ ,,
No. 5-minute stops	4 at 70, 50, 40, 30 feet	4 at 66, 54, 40, 30 feet.
No. 10 ,, ,,	2 at 20, 10 feet	2 at 20, 10 feet.

Monday 27th August,

H.M.S. *Spanker*, off Loch Riddon.

Thirty fathoms of water were obtained. Mr Catto was the diver in the morning. The pumps used were Nos. 3604 and 3593. Six men were told off for each pump, in reliefs of 5 minutes. Details of the descent:

Time	Remarks
11.22	Glass screwed up. Depth by lead line 30½ fathoms.
11.23¼	Diver under water.
11.23½	,, down 50 feet.
11.23¾	,, ,, 70 ,,
11.24¼	,, ,, 110 ,,
11.24½	,, ,, 150 ,,
11.24¾	,, ,, 180 ,, on the bottom. 1 min. 30 secs. in descending. Revolutions averaged 32 per min., but fell to 24 for a short time, owing to the great exertions that were necessary to keep the pumps going at the higher speed. Diver quite comfortable while moving about on the bottom.
11.36¾	Diver called up.
11.38½	,, started up.
11.39¼	,, at 160 feet.
11.39¾	,, ,, 140 ,,
11.40¼	,, ,, 120 ,,
11.41	,, ,, 100 ,,
11.41½	,, ,, 70 ,, 1st stop. Diver employed in gymnastic exercises. One pump stopped.
11.46½	Diver at 50 feet. 2nd stop.
11.51½	,, ,, 40 ,, 3rd stop.
11.56½	,, ,, 30 ,, 4th stop.
12.1½	,, ,, 20 ,, 5th stop.
12.11½	,, ,, 10 ,, 6th stop. There were no ill-effects. Water jackets gained 20 degrees F.
12.22½	Diver called up.
12.23¾	Glass off.

Afternoon. Lieutenant Damant.

2.14½	Screwed up glass.
2.15¾	Diver under water.
2.16	,, down 70 feet.
2.16½	,, ,, 120 ,,
2.16¾	,, ,, 160 ,,
2.17	,, ,, 186 ,, on the bottom. 1 minute 20 seconds in descending. Revolutions averaged 30 per minute.
2.29	Diver called up.
2.30	,, started up.
2.31	,, at 170 feet.
2.33	,, ,, 120 ,, Diver stopped 1¼ minutes.
2.33½	,, ,, 70 ,, 1st stop.
2.38½	,, ,, 50 ,, 2nd ,,

Time	Remarks
2.43½	Diver at 40 feet. 3rd stop.
2.48½	,, ,, 30 ,, 4th ,,
2.53½	,, ,, 20 ,, 5th ,,
3.3½	,, ,, 10 ,, 6th ,,
3.13½	,, called up.
3.15¼	Glass off. There were no ill-effects. Later in the afternoon the pumps were tested at different temperatures of the water jacket, to see how the leakage was affected.

Tuesday, 28th August.

In the same locality, Lieutenant Damant made a second descent in 30 fathoms in order to obtain samples of the air in the helmet. The pumps used were Nos. 3588 and 3592 :

Time	Remarks
10.18½	Diver under water.
10.20¼	,, on the bottom, 1 minute 40 seconds in going down.
10.34½	,, started up.
11.21½	Glass off. Whilst on the bottom, diver took two samples whilst at rest. There was a distinct tide on the bottom, which affected the diver.

Analysis of Samples.

No. of sample	CO_2 per cent.	O_2 per cent.	CO_2 production in cubic feet per minute
1st	·32	20·86	·025
2nd	·50	20·43	·041 (? tide)

In the afternoon Mr Catto was in the dress. Pumps Nos. 3588 and 3592 were used :

Time	Remarks
2.17	Glass screwed up.
2.18¼	Diver down 60 feet.
2.19	,, ,, 100 ,,
2.19¾	,, ,, 180 ,, on the bottom. The diver took down with him a wire hawser to shackle on to a sinker.
2.31¾	Diver called up, but could not come up as he was foul, until—
2.48½	,, started up.
2.50½	,, at 140 feet.
2.53	,, ,, 100 ,, 1st stop.
2.56	,, ,, 80 ,, 2nd ,,
3.1	,, ,, 60 ,, 3rd ,,
3.7	,, ,, 50 ,, 4th ,,
3.12	,, ,, 40 ,, 5th ,,
3.22	,, ,, 30 ,, 6th ,,
3.37	,, ,, 20 ,, 7th ,,
3.52	,, ,, 15 ,, 8th ,,
4.0	,, ,, 10 ,, 9th ,,
4.18½	,, on the surface.

Mr Catto attempted to shackle a hawser on to the sinker. He found the sinker without the slightest difficulty, and then, having tied his distance line to it, went back to the hawser. He found this in bights, and he seems to have got within the coils, and in trying to find the end of the wire to have fouled his life line. When called up he could not get away, and it was 20 minutes before he could clear himself. In all he was down 28¾ minutes in 30 fathoms of water. The rate of the pump could not be kept up above 24 revolutions per minute, and the supply of air was not adequate to his exertions to free himself, so that he was almost overcome by the excess of CO_2. On account of his long exposure during heavy work, great care was taken in decompressing him, 1½ hours being allowed. There were no ill-effects.

Thursday, 30th August,

H.M.S. *Spanker*, off Loch Riddon.

Mr Catto made another descent under the same conditions, and shackled on the hawser to the sinker in 4 minutes after reaching the bottom. The revolution of the pump averaged 24 to 30 per minute. The day was very bright, with the sun shining on the water, so that the diver saw with comparative ease in the water.

In the afternoon Lieutenant Damant, at the same depth, took three samples of the air in the helmet, and the pumps were tested at 180 feet pressure. He suffered from no ill-effects:

	Mr Catto	Lieutenant Damant
Time of descent	3 minutes	1 min. 20 secs.
,, on the bottom	12¾ ,,	13 minutes.
,, of ascent	46¼ ,,	46¼ ,,
No. 5-minute stops	4 at 70, 50, 40, 30 feet	4 at 70, 50, 40, 30 feet.
,, 10 ,, ,,	2 at 20, 10 feet	2 at 20, 10 feet.

Analysis of Samples obtained by Lieutenant Damant.

Per cent.	First sample	Second sample	Third sample
CO_2 … … …	·43	·39	·36
O_2 … … …	20·56	20·52	20·47
Deficiency of oxygen … …	·48	·52	·57
CO_2 produced in cubic feet per minute	·035	·029	·027

Friday, 31st August,

H.M.S. *Spanker* moved down to the entrance of Loch Striven, where 35 fathoms of water could be obtained.

In the morning Lieutenant Damant was the diver. Pumps Nos. 2593, 3604 and 3592 were tested and used. Six hands were told off for each pump in reliefs of 5 minutes:

Time	Remarks
11.8	Glass screwed up.
11.8¼	Diver under water.
11.9	,, down 80 feet.
11.9¼	,, ,, 120 ,,
11.9½	,, ,, 150 ,,
11.9¾	,, ,, 180 ,,
11.10¼	,, ,, 200 ,,
11.10½	,, ,, 216 ,, on the bottom. Revolutions kept at 30 per minute, and the diver had a good supply of air.
11.13½	Diver took samples seated on the shot at the bottom of the rope.
11.15½	,, called up.
11.16¼	,, started up.
11.17	,, at 190 feet.
11.18	,, ,, 110 ,, Diver stopped to blow off sampling tube.
11.20½	,, ,, 90 ,, 1st stop.
11.23½	,, ,, 70 ,, 2nd ,,
11.28½	,, ,, 52 ,, 3rd ,,
11.33½	,, ,, 42 ,, 4th ,,
11.39½	,, ,, 32 ,, 5th ,,
11.44½	,, ,, 22 ,, 6th ,,
11.54½	,, ,, 11 ,, 7th ,,
12.4½	,, called up.

There was no light on the bottom, which was of soft mud. The depth by the shot rope was 210 feet. Pressure was 93½ lbs. The gauge showed a pressure of 216 feet of fresh water with the pumps stopped, and 220 feet whilst they were heaving. The actual depth, as carefully measured on the shot rope against the ship's standard measure, was just over 35 fathoms, 210 feet.

In the afternoon Mr Catto made the same descent, and reached 35 fathoms. He found that the air supply was more than ample. He walked out to the end of his distance line, and then took a sample of the air in his helmet:

Time	Remarks
2.12	Screwed up glass. Same pumps as last.
2.12¾	Diver under water.
2.14¾	,, on the bottom. Revolutions reduced to 24, as the diver found the supply too much. He proceeded to the end of his distance line before taking his sample.
2.20½	Diver started up.
2.27¾	,, at 90 feet. 1st stop.
2.30½	,, ,, 70 ,, 2nd ,,
2.35¾	,, ,, 50 ,, 3rd ,,
2.40¾	,, ,, 40 ,, 4th ,,
2.45¾	,, ,, 30 ,, 5th ,,
2.50¾	,, ,, 20 ,, 6th ,,
3.0¾	,, ,, 10 ,, 7th ,,
3.10¾	,, called up.

Analysis of Samples.

	Lieut. Damant	Mr Catto
CO_2 per cent.	·14	·53
O_2 ,, ,, ...	20·89	20·34
Deficiency of O_2 per cent	·15	·70

Monday, 3rd September.

Experiments on rest and measured work were carried out, by means of an arrangement of rope and pulleys by which the diver on the bottom raised and lowered a 56 lb. weight suspended in view of those on deck. The heavy rope and blocks used caused great friction and resistance.

Time	Remarks	
2.26	Diver, Mr Catto, descended.	
2.27¼	,, on bottom, 142 feet.	
2.31	,, took sample sitting on the shot.	(No. 1.)
	Two pumps at 30 revolutions per minute.	
	Raised the weight 4 times 5 feet, at the rate of one lift per minute.	
2.36	,, took sample.	(No. 2.)
	Raised weight 7 times 5 feet in 5½ minutes.	
2.42	,, took sample.	(No. 3.)
2.45	,, started up.	
3.23	,, on surface, no ill-effects.	
3.3½	,, Lieutenant Damant, started down.	
3.4½	,, down 100 feet.	
3.5	,, on bottom, 139 feet.	
4.0	,, took sample sitting on the shot.	(No. 4.)
	Two pumps at 26 revolutions.	
	Raised weight 5 times in 1¾ minutes.	
4.3	,, took sample.	(No. 5.)
	Raised weight 3 feet 18 times in 6¼ minutes.	
4.10	,, took sample. Pump 24 revolutions.	(No. 6.)
4.13½	,, started up.	
4.52½	,, at the surface. No ill-effects.	

Analysis of Samples.

CO_2	·30	per cent.	Mr Catto. Sample No. 1.
O_2	20·72	,,	
CO_2	·70	,,	,, ,, No. 2.
O_2	20·29	,,	
CO_2	·71	,,	,, ,, No. 3.
O_2	20·23	,,	
CO_2	·18	,,	Lieutenant Damant. Sample No. 4.
O_2	20·73	,,	
CO_2	·73	,,	,, ,, No. 5.
O_2	20·12	,,	
CO_2	·81	,,	,, ,, No. 6.
O_2	20·36	,,	

Tuesday, 4th September.

The *Spanker* was anchored in six fathoms of water, and experiments were made on the bottom by Dr Haldane, Lieutenant Damant, and Mr Catto on the risks of blowing up. After being compressed in the air chamber to teach them to open their Eustachian tubes, Lieutenant and Commander E. V. F. R. Dugmore, Lieutenant G. N. Henson, Jack Haldane (age 13) all made descents in six fathoms of water. This was the first time that these had ever dived in a diving dress, which illustrates the usefulness of the re-compression chamber in the practical teaching of divers.

Wednesday, 5th September.

Exhaustive tests were made as to the leakage of the pumps and composition of the air, with the water jackets at various temperatures. The results are embodied in the Report. These experiments concluded the work undertaken for the Committee.

APPENDIX III.

We give here some illustrative protocols of certain important animal experiments.

1. *Comparison of stage* (93 *minutes*) *and uniform* (100 *minutes*) *decompression after* 2 *hours exposure at* 75 *lbs.*

(*a*) 15.3.07. Goats 3, 4, X A, XI A, XVI A, XIX A, XXV A.

Started up	10.23	
Reached 75 lbs.	11.3	
Started from 75 lbs.	12.43	Stage decompression. Total = 93 mins.
,, 31½ ,,	12.49	
,, 27 ,,	12.59	
,, 22 ,,	1.14	
,, 18 ,,	1.29	
,, 13½ ,,	1.44	
,, 9 ,,	1.59	
,, 4½ ,,	2.14	
Reached 0 lbs.	2.16	

XIX A had bends left hind-leg at 2.20. X A passed urine not frothy at 2.27. Rest nil.

(*b*) 18.3.07. Goats XII A, XIII A, XV A, XVIII A, XX A, XXI A, XXIII A.

Started up	10.53	
Reached 75 lbs.	11.35	
Started from 75 lbs.	1.14	Stage decompression. Total = 93 mins.
,, 31½ ,,	1.20	
,, 27 ,,	1.30	
,, 22 ,,	1.45	
,, 18 ,,	2.0	
,, 13½ ,,	2.15	
,, 9 ,,	2.30	
,, 4½ ,,	2.45	
Reached 0 lbs.	2.47	

XX A urine no froth at 2.54. XIII A at 2.55 seemed uneasy in hind-legs and lay down but nothing definite; at 3.0 right hind-leg slight limp and foot-drop; foot-drop very marked at 3.10 and could hardly walk; alright at 4.0. Rest nil.

(*c*) 14.3.07. Goats XXIV A, XXVI A, XXVII A, XXVIII A, XXIX A.

Started up	10.35	
Reached 75 lbs.	11.14	
Started from 75 lbs.	12.55	Stage decompression. Total = 93 mins.
,, 31½ ,,	1.1	
,, 27 ,,	1.11	
,, 22½ ,,	1.26	
,, 18 ,,	1.41	
,, 13½ ,,	1.56	
,, 9 ,,	2.11	
,, 4½ ,,	2.26	
Reached 0 lbs.	2.28	

XXIV A showed bends left fore-leg during decompression from 4½ to 0; XXVII A passed urine no froth at 2.32; XXVIII A bends left hind-leg at 2.40. Rest nil.

(*d*) 12.3.07. Goats XXIV A, XXVI A, XXVII A, XXVIII A, XXIX A.

Started up	12.53	
Reached 75 lbs.	1.1	
Started from 75 lbs.	3.0	Uniform decompression. Total = 100 mins.
Reached 60 lbs.	3.20	
,, 45 ,,	3.40	
,, 30 ,,	4.0	
,, 15 ,,	4.20	
,, 0 ,,	4.40	

XXIX A bleated at about 1 lb., gnawed side, lay down; both fore-legs completely paralysed and hind-legs unsteady; kept head bent round on left side; bleated continually; no dyspnoea till 4.55, when it was moderate; seemed like to die. At 5.10 no dyspnoea, stopped bleating, could just stand. Could walk at 5.35. XXVII A passed very frothy urine at 4.41; both hind-legs bad bends at 4.50, right fore-leg at 5.10. XXIV A left hind-leg partial foot-drop and bends at 4.50; left fore-leg bends at 5.0. XXVIII A bad bends right fore-leg, won't stand up; at 5.0 could not stand, had constant nystagmus, bleated; at 5.10 left hind-leg bad bends, nystagmus stopped, no bleating; walked very badly at 5.30. XXVI A no symptoms.

(*e*) 19.3.07. Goats 3, 4, X A, XI A, XVI A, XIX A, XXV A.

Started up	10.30	
Reached 75 lbs.	11.14	
Started from 75 lbs.	12.52	Uniform decompression. Total = 100 mins.
Reached 60 lbs.	1.12	
,, 45 ,,	1.32	
,, 30 ,,	1.52	
,, 15 ,,	2.12	
,, 0 ,,	2.32	

XVI A came out with bad bends left hind and right fore-legs; could hardly walk and kept head twisted round to left; much better at 2.50. XIX A urine at 2.34 full of froth; bends right fore-leg. X A began bleating at 2.38 but showed nothing till 2.44 when he had complete foot-drop right fore-leg and bends left hind-leg; at 2.50 right fore-leg paralysed, could not stand up, left fore-leg also weak; urine at 2.50 a little froth. XXV A cried out a bit, belly very tight, refuses to move, evidently far from well: died between 8 and 8.30 a.m. next day: a good many bubbles in right heart. Rest nil.

(*f*) 20.3.07. Goats XII A, XIII A, XV A, XVIII A, XX A, XXI A, XXIII A.

Started up	11.5	
Reached 75 lbs.	11.47	
Started from 75 lbs.	1.26	Uniform decompression. Total = 100 mins.
Reached 60 lbs.	1.46	
,, 45 ,,	2.6	
,, 30 ,,	2.26	
,, 15 ,,	2.46	
,, 0 ,,	3.6	

A goat unknown aborted two foetuses 2 in. long; they were quite warm when found, so probably during decompression. XXIII A very frothy urine at 3.8. XIII A dyspnoea, both hind-legs dragging; at 3.25 lying down, moaning bleat, tongue and lips getting cyanosed, dyspnoea not violent. Made sure it was going to die, but at 3.55 it got up and showed only bends right fore-leg and weakness in both hind-legs. At 5.0 seemed all right. Rest nil.

(2) *The effects of a sudden drop of* 51 *lbs. in different parts of the scale of absolute pressure.*

(*a*) 26.3.07. Goats XXIV A, XXVI A, XXVII A, XXVIII A, XXIX A.

Started up	10.0	
Reached 75 lbs.	10.46	
Left 75 lbs.	1.23	
Reached 24 lbs.	1.24½	
Left 24 lbs.	2.25	
,, 14 ,,	2.55	
,, 8 ,,	3.18½	
Reached 0 lbs.	3.21½	Total=118½ mins.

No symptoms during decompression. XXVIII A passed frothy urine at 3.23; at 3.31 had bad bends, evidently very uneasy generally; better at 4.0. XXIX A urine no froth at 3.31. Rest nil. (The immediate object of the experiment having been attained, an unwise quickening of the end of decompression gave XXVIII A bad bends.)

(*b*) 23.5.07. Goats 7, 9, XXX A, XXXII A, XXVII.

Start up ...	9.55	
Reach 75 lbs.	10.35	
Left 75 lbs.	1.15	
Reach 24 lbs.	1.15.40″	
Left 24 lbs.	2.15	
,, 14 ,,	2.45	
,, 8 ,,	3.5	
,, 4 ,,	3.25	Total=131 mins.

No symptoms during decompression. 7 limped right hind-leg on coming out; urine 3.30 no froth. 9 right hind-leg bends at 3.35. XXXII A urine 3.35 no froth. Rest nil.

(*c*) 27.3.07. Goats XXIV A, XXVI A, XXVII A, XXVIII A, XXIX A.

Started up	11.0
Reached 51 lbs.	11.30
Left 51 lbs.	2.16
Reached 0 lbs.	2.20¼

XXVIII A very unsteady on hind-legs at 2.25, passed urine full of froth; legs gave way; at 2.30 lying down grunting, constant nystagmus, mucous membranes not pale; at 2.38 respiratory movements almost stopped; died 2.44 p.m. Ordinary moderate bubbling. XXVII A bends left fore-leg at 2.27, bad; a little left next day. XXIV A left hind-leg bends at 2.29; had pretty marked dyspnoea at 2.40. XXIX A urine 2.40 no froth, seemed uneasy, kept lying down but could make out nothing definite. XXVI A no symptoms.

(*d*) 24.5.07. Goats 7, 9, XXVII A, XXX A, XXXII A, XXVII.

Started up	9.51
Reached 51 lbs.	10.15
Started down	1.3
Reached 0 lbs.	1.7

XXX A, urine 1.10 much froth, no symptoms. XXVII A, bad bends left fore-leg, jumpy hind-legs. 7, bends right fore-leg. 9, bends right fore-leg; slight dyspnoea, bends bad, both hind-legs wobbly; dyspnoea gone by 1.40 and legs alright. XXXII A bleating, won't stand up, dyspnoea; died 1.34 p.m. Bad general bubbling. XXVII bends right fore-leg.

(*e*) 5.6.07. Goats XII A, XVI A, XXIII A.

Started up	9.52
Reached 45 lbs.	10.14
Left 45 lbs.	12.3
Reached 0 lbs.	12.3.33″
,, −6 lbs.	12.10

XVI A uneasy at −5 lbs., paraplegic at 12.10, struggling and bleating, dyspnoea. XII A bends right fore-leg 12.19, bleating at 12.28. XXIII A tried to get up at 12.25 but failed once; then got up, right hind-leg paralysed; both gone just afterwards, could just crawl across tank; dyspnoea at 12.28. Raised pressure to atmospheric and opened tank at 1.10. XII A got up and seemed alright. XXIII A and XVI A lay log-like, conscious, breathing slightly and slowly. At 1.40 XXIII A could rest on fore-legs, hind-legs completely paralysed, ate hay; seemed pretty well except for paraplegia at 4.0. (Condition did not improve and it was killed six days later.) XVI A died at 3.20 p.m. A few small bubbles in right auricle and right femoral vein.

(*b*) 12 . 6 . 07. Goats 7, XXIV A, XXIX A, XXX A.

Started up	9.55
Reached 39 lbs.	10.13
Left 39 lbs.	12.4.20″
Reached 0 lbs.	12.4.50″
Left 0 lbs.	12.6.10″
Reached − 6 lbs.	12.11.5″

XXX A bends right fore-leg at 12.13, dyspnoea at 12.30. XXIX A bends right fore-leg at 12.14, lay down, dyspnoea at 12.24. 7 lay down, dyspnoea at 12.14. XXIV A no symptoms. Raised pressure to normal and opened up at 1.15. 7 showed bends left fore-leg and had slight dyspnoea. XXX A seemed alright. XXIV A and XXIX A were very quiet but no definite symptoms. All alright at 3.30.

APPENDIX IV.

TABLE I.

Stoppages during the ascent of a diver after ordinary limits of time from surface.

Depth		Pressure — Pounds per square inch	Time from surface to beginning of ascent	Approximate time to first stop	Stoppages in minutes at different depths*						Total time for ascent in mins.
Feet	Fathoms				60 ft.	50 ft.	40 ft.	30 ft.	20 ft.	10 ft.	
0–36	0–6	0–16	No limit ...	—	—	—	—	—	—	—	0–1
36–42	6–7	16–18½	Over 3 hours	1	—	—	—	—	—	5	6
42–48	7–8	18½–21	Up to 1 hour	—	—	—	—	—	—	—	1½
			1–3 hours ...	1½	—	—	—	—	—	5	6½
			Over 3 hours	1½	—	—	—	—	—	10	11½
48–54	8–9	21–24	Up to ½ hour	—	—	—	—	—	—	—	2
			½–1½ hours ...	2	—	—	—	—	—	5	7
			1½–3 hours ...	2	—	—	—	—	—	10	12
			Over 3 hours	2	—	—	—	—	—	20	22
54–60	9–10	24–26½	Up to 20 mins.	—	—	—	—	—	—	—	2
			20–45 mins. ...	2	—	—	—	—	—	5	7
			¾–1½ hours ...	2	—	—	—	—	—	10	12
			1½–3 hours ...	2	—	—	—	—	5	15	22
			Over 3 hours	2	—	—	—	—	10	20	32
60–66	10–11	26½–29½	Up to ¼ hour	2	—	—	—	—	—	—	2
			¼–½ hour ...	2	—	—	—	—	—	5	7
			½–1 hour	2	—	—	—	—	3	10	15
			1–2 hours ...	2	—	—	—	—	5	15	22
			2–3 hours ...	2	—	—	—	—	10	20	32
66–72	11–12	29½–32	Up to ¼ hour	2	—	—	—	—	—	2	4
			¼–½ hour ...	2	—	—	—	—	3	5	10
			½–1 hour ...	2	—	—	—	—	5	12	19
			1–2 hours ...	2	—	—	—	—	10	20	32
72–78	12–13	32–34½	Up to 20 mins.	2	—	—	—	—	—	5	7
			20–45 mins. ...	2	—	—	—	—	5	10	17
			¾–1½ hours ...	2	—	—	—	—	10	20	32
78–84	13–14	34½–37	Up to 20 mins.	2	—	—	—	—	—	5	7
			20–45 mins. ...	2	—	—	—	—	5	15	22
			¾–1¼ hours ...	2	—	—	—	—	10	20	32
84–90	14–15	37–40	Up to 10 mins.	2	—	—	—	—	—	3	5
			10–20 mins. ...	2	—	—	—	—	3	5	10
			20–40 mins. ...	2	—	—	—	—	5	15	22
			40–60 mins. ...	2	—	—	—	3	10	15	30
90–96	15–16	40–42½	Up to 10 mins.	3	—	—	—	—	—	3	6
			10–20 mins. ...	2	—	—	—	—	3	5	10
			20–35 mins. ...	2	—	—	—	—	5	15	22
			35–55 mins. ...	2	—	—	—	3	10	15	30
96–108	16–18	42½–48	Up to 15 mins.	3	—	—	—	—	3	5	11
			15–30 mins. ...	3	—	—	—	3	7	10	23
			30–40 mins. ...	3	—	—	—	5	10	15	33
108–120	18–20	48–53½	Up to 15 mins.	3	—	—	—	2	3	7	15
			15–25 mins. ...	3	—	—	—	5	5	10	23
			25–35 mins. ...	3	—	—	—	5	10	15	33
120–132	20–22	53½–59	Up to 15 mins.	3	—	—	—	2	5	7	17
			15–30 mins. ...	3	—	—	—	5	10	15	33
132–144	22–24	59–64½	Up to 12 mins.	3	—	—	—	3	5	5	16
			12–25 mins. ...	3	—	—	2	5	10	12	32
144–156	24–26	64½–70	Up to 10 mins.	3	—	—	—	3	5	5	16
			10–20 mins. ...	3	—	—	2	5	10	12	32
156–168	26–28	70–75	Up to 10 mins.	3	—	—	2	3	5	5	18
			10–16 mins. ...	3	—	2	3	5	7	10	30
168–180	28–30	75–80½	Up to 9 mins.	3	—	—	2	3	5	5	18
			9–14 mins. ...	3	—	2	3	5	7	10	30
180–192	30–32	80½–86	Up to 13 mins.	3	—	2	3	5	7	10	30
192–204	32–34	86–91½	Up to 12 mins.	3	2	2	3	5	7	10	32

* During each stoppage the diver should continue to move his arms and legs.

TABLE II.

Stoppages during the ascent of a diver after delay beyond the ordinary limits of time from surface.

Depth		Pressure — Pounds per square inch	Time from surface to beginning of ascent	Approximate time to first stop	Stoppages in minutes at different depths								Total time for ascent in mins.
Feet	Fathoms				80 ft.	70 ft.	60 ft.	50 ft.	40 ft.	30 ft.	20 ft.	10 ft.	
60–66	10–11	26½–29½	Over 3 hours	2	—	—	—	–	—	—	10	30	42
66–72	11–12	29½–32	2–3 hours ...	2	—	—	—	—	—	—	10	30	42
			Over 3 hours	2	—	—	—	—	—	—	20	30	52
72–78	12–13	32–34½	1½–2½ hours	2	—	—	—	—	—	—	20	25	47
			Over 2½ hours	2	—	—	—	—	—	—	30	30	62
78–84	13–14	34½–37	1¼–2 hours ...	2	—	—	—	—	—	—	15	30	47
			2–3 hours ...	2	—	—	—	—	—	5	30	30	67
			Over 3 hours	2	—	—	—	—	—	10	30	35	77
84–90	14–15	37–40	1–1½ hours ...	2	—	—	—	—	—	5	15	25	47
			1½–2½ hours...	2	—	—	—	—	—	5	30	35	72
			Over 2½ hours	2	—	—	—	—	—	20	35	35	92
90–96	15–16	40–42½	1–1½ hours ...	2	—	—	—	—	—	5	15	30	52
			1½–2½ hours...	2	—	—	—	—	—	10	30	35	77
			Over 2½ hours	2	—	—	—	—	—	30	35	35	102
96–108	16–18	42½–48	40–60 minutes	2	—	—	—	—	—	10	15	20	47
			1–2 hours ...	2	—	—	—	—	5	15	25	35	82
			Over 2 hours	2	—	—	—	—	15	30	35	40	122
108–120	18–20	48–53½	35–60 minutes	2	—	—	—	—	5	10	15	25	57
			1–2 hours ...	2	—	—	—	—	10	20	30	35	97
			Over 2 hours	2	—	—	—	—	30	35	35	40	142
120–132	20–22	53½–59	½–¾ hours ...	3	—	—	—	—	5	10	15	20	53
			¾–1½ hours ...	3	—	—	—	5	10	20	30	30	98
			Over 1½ hours	3	—	—	—	15	30	35	40	40	163
132–144	22–24	59–64½	25–45 minutes	3	—	—	—	3	5	10	15	25	61
			¾–1½ hours ...	3	—	—	—	10	10	20	30	35	108
			Over 1½ hours	3	—	—	—	30	30	35	40	40	178
144–156	24–26	64½–70	20–35 minutes	3	—	—	—	3	5	10	15	20	56
			35–60 minutes	3	—	—	—	7	10	15	30	30	95
			Over 1 hour	3	—	—	20	25	30	35	40	40	193
156–168	26–28	70–75	16–30 minutes	3	—	—	—	3	5	10	15	20	56
			½–1 hour ...	3	—	—	3	10	10	15	30	30	101
			Over 1 hour	3	—	5	25	25	30	35	40	40	203
168–182	28–30	75–80½	14–20 minutes	3	—	—	—	3	3	7	10	15	41
			20–30 minutes	3	—	—	2	2	3	10	15	25	60
			½–1 hour ...	3	—	3	3	7	10	20	30	35	111
			Over 1 hour	3	—	15	25	30	30	35	40	40	218
182–194	30–32	80½–86	13–20 minutes	3	—	—	—	3	3	7	15	15	46
			20–30 minutes	3	—	—	3	3	5	10	15	25	64
			½–1 hour ...	3	—	3	5	10	12	20	30	35	118
			Over 1 hour	3	5	20	25	30	30	35	40	40	228
194–206	32–34	86–91½	12–20 minutes	3	—	—	3	3	5	7	10	20	51
			20–30 minutes	3	—	3	3	3	5	10	20	20	67
			½–1 hour ...	3	3	3	5	10	15	20	30	35	124
			Over 1 hour	3	15	20	25	30	30	35	40	40	238

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THE EFFECTS OF LOW ATMOSPHERIC PRESSURES ON RESPIRATION.

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It is well known that exposure to the low atmospheric pressures met with at high altitudes produces a variety of respiratory and other disturbances. The most prominent of these are that muscular exertion produces undue hyperpnœa: that sleep is apt to be disturbed by irregular breathing: and that attacks of nausea, headache, and great depression ("mountain sickness") are apt to occur. In very high ascents in balloons, consciousness is often lost quite suddenly, and death may easily result.

Paul Bert[1] was the first to furnish clear experimental evidence that the main cause of these symptoms is the low partial pressure of oxygen in the air breathed, and consequent incomplete saturation of the blood with oxygen. Subsequent investigations, for an account of which we may refer to the books of H. von Schrötter[2], and of Zuntz, Loewy, Müller, and Caspari[3], have, when correctly interpreted, confirmed Bert's main conclusions, although, as will be seen below, many points are still far from clear, and particularly the part played by CO_2 in the regulation of respiration at low pressures.

The physiological effects of low barometric pressures evidently depend on the partial pressure of oxygen, and possibly also of CO_2, in the air of the lung alveoli; and a knowledge of these is essential to a proper understanding of respiration at low pressures. The present investigation has been devoted almost entirely to following the changes in the partial pressure of CO_2 and O_2 in alveolar air at low pressures, in conjunction with the symptoms produced.

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[1] Paul Bert. La Pression Barométrique, 1878.

[2] H. von Schrötter. *Der Sauerstoff in Luftdruckerkrankungen*, Berlin, 1906. This book contains a very interesting account and discussion of recent very high balloon ascents with the help of oxygen.

[3] Zuntz, Loewy, Müller, and Caspari. *Höhenklima und Bergwanderungen*, Berlin, 1906.

It was shown by Haldane and Priestley[1] that under normal conditions the breathing is so regulated as to maintain in each individual a practically constant partial pressure (about 40 mm. of mercury) of CO_2 in the alveolar air, in spite of great changes in the rate of respiratory exchange or in the composition or pressure of the inspired air. So delicate is the regulation that, other things being equal, a rise of 1 mm. in the CO_2 pressure increases the breathing about 80 %. This law held good when the alveolar oxygen pressure was increased by breathing air at increased pressure, or containing an increased percentage of oxygen. When the alveolar oxygen pressure was greatly diminished, however, as by breathing air containing less than about 14 % of oxygen, the law no longer held good. Thus with an alveolar oxygen pressure reduced from the normal of about 103 mm. of mercury to 31 mm. there was great hyperpnœa with other urgent symptoms, and the alveolar CO_2 pressure had fallen from 40 to 25 mm.[2]

The hyperpnœa caused by a great fall in the oxygen pressure of the air breathed has of course been long familiar to physiologists, and deficiency of oxygen in the alveolar air was till recently regarded as a factor in the regulation of breathing under even normal conditions. An accurate study of the effects on respiration of deficient oxygen pressure has hitherto been wanting, however.

Zuntz and his associates have rightly laid great stress on the importance of the alveolar gas-pressures in connection with the effects of low pressures, and they have published numerous data relating to this point. Their method was to assume a certain volume (140 c.c.) calculated by Loewy[3] from various data, for the "dead space" of the respiratory passage, and to calculate the composition of the alveolar air from this assumed volume, and the observed composition of the expired air and depth of breathing. This method seems from the results to be uncertain, and this uncertainty has, we think, misled the observers in question to some extent. A number of experiments are summarised in Tables X to XVI of the book by Zuntz, Loewy, Caspari and Müller. In Table X, for example, the calculated alveolar CO_2 pressure is 33·1 mm. at Berlin (barometer 756 mm.), 44·5 at Brienz (715 mm.) and 34·8 mm. on Monte Rosa (439 mm.). It seems very improbable that these results could be correct. Table XVI gives results obtained in a pneumatic chamber at Berlin. At a pressure of about 480 mm. the first two of the five individuals investigated gave alveolar CO_2 pressures considerably above the maximum (44·5 mm.) directly determined by Fitzgerald and Haldane, who examined a large number of individuals[1]. Such a combination of alveolar gas pressures as the extremely high value of 50·9 mm. for CO_2 with the extremely low one of 32·8 for oxygen seems specially improbable in view of all our results. In any case the value assumed for the dead space in these calculations must be uncertain, so that the method can only give doubtful results for any individual. If, however, the results for all the persons experimented on be averaged, general conclusions can be drawn which are probably near the truth. The average, for instance, shows a considerably lower alveolar CO_2 pressure on Monte Rosa than at a normal atmospheric pressure.

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[1] This *Journal*, XXXII. p. 225. 1905.

[2] *Loc. cit.* p. 235.

[3] *Pflüger's Archiv*, LVIII. p. 416. 1894.

Our experiments were made in the large steel chamber recently presented to the Lister Institute by Dr Ludwig Mond. This chamber, which is described more fully elsewhere[2], is sufficiently roomy to enable three persons to work comfortably and carry out the necessary analyses, etc. Any desired negative pressure can easily be obtained and kept so steady that in spite of ample ventilation by a powerful pump there is no difficulty in carrying out the analyses. In several of the later experiments we had the co-operation of Mr Ogier Ward, who, in particular, acted as subject and made the analyses, in the very long experiment described below. The composition of the alveolar air was investigated by the direct method of Haldane and Priestley[3], the partial pressure of O_2 and CO_2 being calculated from the results of the analyses and the readings of a mercurial barometer in the chamber, after allowing for the pressure of aqueous vapour (46 mm. of mercury) in the saturated alveolar air. Thus if the percentage of O_2 in the (dry) alveolar air was found by analysis to be 11·00 %, and the barometric pressure was 400 mm., the oxygen pressure in the alveolar air was $(400 - 46) \times \frac{11}{100} = 38{\cdot}5$ mm. Two samples (one at the end of inspiration and one at the end of expiration) were taken for each determination.

The analyses were made inside the steel chamber with the gas-analysis apparatus described by Haldane in the *Journal of Hygiene*, vol. VI. p. 74, 1906, and the samples were almost always taken directly into the gas-burette, the arrangement being that used by Fitzgerald and Haldane[1]. Accurate determinations could thus be made very easily and quickly. For absorbing the oxygen we often used the hydrosulphite solution recommended by Durig[2], and found it to be an admirable and cleanly substitute for pyrogallic acid solution, though not so lasting.

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[1] This *Journal*, XXXII. p. 486. 1905.

[2] Boycott, Damant, and Haldane. *Journal of Hygiene*, VIII. p. 377. 1908.

[3] This *Journal*, XXXII. p. 226. 1905.

Before discussing the results of the low pressure experiments we must refer to a possible source of fallacy in our experiments. At the outset of the investigation we found that although under ordinary conditions the alveolar CO_2 pressure remains very constant, as stated by Haldane and Priestley, yet any considerable changes in the external temperature produce distinct alterations. It was observed by Haldane in an experiment where the body temperature was allowed to rise in a hot and moist atmosphere that the alveolar CO_2 pressure fell considerably[3]. This was at the time attributed entirely to the rise in body temperature; but further observations have shown us that warmth or cold causes a slight fall or rise in the alveolar CO_2 pressure without any rise or fall of the internal (rectal) temperature. These variations are not large, but are sufficient to make it necessary to take into careful consideration the circumstances of temperature when any small changes in the alveolar CO_2 pressure are under examination.

Time	Alveolar CO_2 per cent.	Remarks
11.23	5·415	
11.5	5·66	In laboratory: air t.° 18·4—19·0°.
12.20	5·62	Average 5·51 per cent. = 39·1 mm. Hg.
12.35	5·335	Rectal temp. at 1.5 was 37·2°.
12.50	5·52	
1.22	6·03	At 1.20 into cold room: air t.° 7·1—8·0°.
1.35	5·93	Average 5·95 per cent. = 42·2 mm. Hg.
1.46	5·90	Rectal temp. at 1.50 was 37·0°.
2.6	5·34	At 1.59 from cold room to laboratory: air t.° 18·0—18·2°.
2.23	5·60	Average 5·49 per cent. = 39·0 mm. Hg.
2.35	5·54	
2.54	5·875	At 2.52 into cold room again: air t.° 7·8°.
3.8	5·825	Average 5·85 per cent. = 41·5 mm. Hg.
3.35	5·46	At 3.25 left cold room: 3.32 into hot room: air t.° 38·0°.
3.48	5·27	Average 5·38 per cent. = 38·2 mm. Hg.
4.4	5·40	Rectal temp. at 4.5 was 37·0°.

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[1] This *Journal*, XXXII. p. 486. 1905.

[2] *Biochem. Zeitsch.* IV. p. 65. 1907. To a solution of 50 grammes of $Na_2S_2O_4$ in 240 c.c. of water add a solution of 30 grammes of NaHO in 40 c.c. of water.

[3] *Journal of Hygiene*, v. p. 503. 1905. Strangely enough, Scott has quite recently proved (This *Journal*, XXXVII. p. 314. 1908) that fall of body temperature also causes (in cats) a *fall* in the alveolar CO_2 pressure.

The phenomenon has been observed in ourselves at intervals during 18 months, and has been found to be qualitatively, if not quantitatively constant. A typical experiment gave the following results. The subject (A. E. B.) was fully clothed. The figures given for the CO_2 represent in each case the average of two samples, one taken at the end of inspiration, the other at the end of expiration. The time indicates the time of taking the first (inspiration) sample of each pair.

A number of further observations on the same subject are summarised in the next table. The figures in each case represent the average of two to eight pairs of samples.

		CO_2			CO_2			CO_2	
Barometer	T°	per cent.	mm. Hg	T°	per cent.	mm. Hg	T°	per cent.	mm. Hg
766	5	6·02	43·3				28	5·34	38·5
756	7·5	5·91	42·0	18·5	5·50	39·0	38	5·38	38·2
752	7	5·86	41·4	16·5	5·52	39·0			
762				15·5	5·80	41·5	23	5·46	39·1
774	5·5	5·58	40·6	16·5	5·22	38·0			
768	9	5·61	40·5	18·2	5·54	40·0	27·5	5·40	39·0
767				15·0	5·69	41·0	24	5·30	39·2

This influence of temperature also seemed to be effective in producing a general seasonal variation in the average normal alveolar CO_2 tension determined at room temperature. A number of determinations made for a variety of purposes seems to show on examination that the alveolar CO_2 tends to a lower level in warm weather. The following figures are averages obtained in the same subject by exactly the same method throughout:

May 1906 (6 days) 41·5, 42·0, 42·7, 40·3, 41·7, 41·9 = 41·7.
June 1906 (7 days) 39·0, 39·0, 41·5, 38·0, 39·4, 40·0, 41·0 = 39·7.
November 1906 (3 days) 39·7, 42·0, 42·2 = 41·3.
July 1907 (7 days) 40·9, 37·6, 39·4, 41·3, 38·6, 39·6, 41·5 = 39·8.
August 1907 (3 days) 38·6, 39·8, 40·4 = 39·6.

The temperature of the air of the laboratory on all the occasions mentioned lay between 14° and 19°. The authors are of opinion from general experience, which it is not possible to express in numerical terms, that this diminution in the alveolar CO_2 is associated with the feeling of warmth of a rather unpleasant kind rather than with any absolute point on the thermometer. It is clear that it has nothing to do with body temperature as measured in the rectum, and is produced by contact of warm air with the face and hands within two or three minutes.

We think indeed that it is one of the physical expressions of the feeling of warmth and slackness, while the rise in the CO_2 tension is associated with the general exhilaration and stimulation produced by cold air.

It is possible that the influence of air temperature on alveolar CO_2 is secondary to alterations in the circulation. An increase in the rate of circulation through the respiratory centre would tend to diminish the difference between the tension of CO_2 in the cells there and in the alveolar air, and therefore to raise the tension in the lung. If we may with propriety infer that a general rise of blood-pressure indicates an increase of circulation through the respiratory centre, the following experiments bear on this question. The blood-pressure was determined by Martin's Riva Rocci apparatus under conditions of rest (sitting still) and gentle activity (walking slowly up and down the laboratory): no difficulty was experienced in obtaining concordant successive readings. The alveolar CO_2 was determined at the same time.

Air temperature	Alveolar CO_2	Blood-pressure (resting)	Remarks
16·5°	5·76, 5·79 = 42·0	101, 102, 101, 100, 99, 101 = 101	In laboratory.
8·3°	6·17, 5·91 = 43·7	107, 109, 109, 109 = 108	In yard.
35·5°	5·50, 5·55 = 40·3	102, 100, 102, 101 = 101	In hot room.
16°, resting	5·83, 5·84, 5·69 = 42·2	104, 107, 106, 104, 104, 105 = 105	In laboratory.
16°, active	6·19, 6·07, 5·93 = 44·3	118, 121, 120 = 120	,,
35°, resting	5·54, 5·48, 5·46 = 39·8	98, 98, 98 = 98	In hot room.
35°, active	5·32, 5·51, 5·42 = 39·5	108, 104, 109, 109 = 107	,,

These results show that the changes of temperature examined may be accompanied by changes in blood-pressure corresponding in sense to the changes in alveolar CO_2 tension. The experiments in the hot room show however that this parallelism may fail and suggest therefore that the two phenomena are associated but not causally related.

The only obvious explanation of the results appears to be that the activity of the respiratory centre is influenced by the varied stimulation of the face, nose etc. by hot and cold air, as well as by the CO_2 pressure in its cells. The alveolar CO_2 pressure is an index of the CO_2 pressure in the respiratory centre and accordingly varies as the sensibility of the centre to CO_2 is influenced by other stimuli.

The results of individual experiments on the alveolar air during exposure to low pressures are given in an Appendix, and will now be briefly summarised and discussed.

We found that in the ordinary short experiments the alveolar CO_2 pressure remained nearly constant until the barometric pressure fell to about 550 mm. or 72 $^0/_0$ of the normal. This is shown in Fig. 1, which represents graphically the results of the first two experiments. At lower pressures there was a fall in the alveolar CO_2 pressure, and this became more and more rapid as the barometric pressure diminished. The law of Haldane and Priestley that during rest under ordinary conditions the alveolar CO_2 pressure remains constant, thus ceased to hold good at less than about 550 mm. of air-pressure. This corresponds to an oxygen pressure of about 116 mm. of mercury, or 15·3 °/₀ of oxygen in dry inspired air at normal pressure. In the first two experiments the alveolar oxygen pressure was not determined; but assuming that the (uncorrected) respiratory quotient was the normal one of about 0·9, the alveolar oxygen pressure at 550 mm. barometric pressure must have been just 62 mm. corresponding to 8·7 °/₀ of oxygen in (dry) alveolar air at

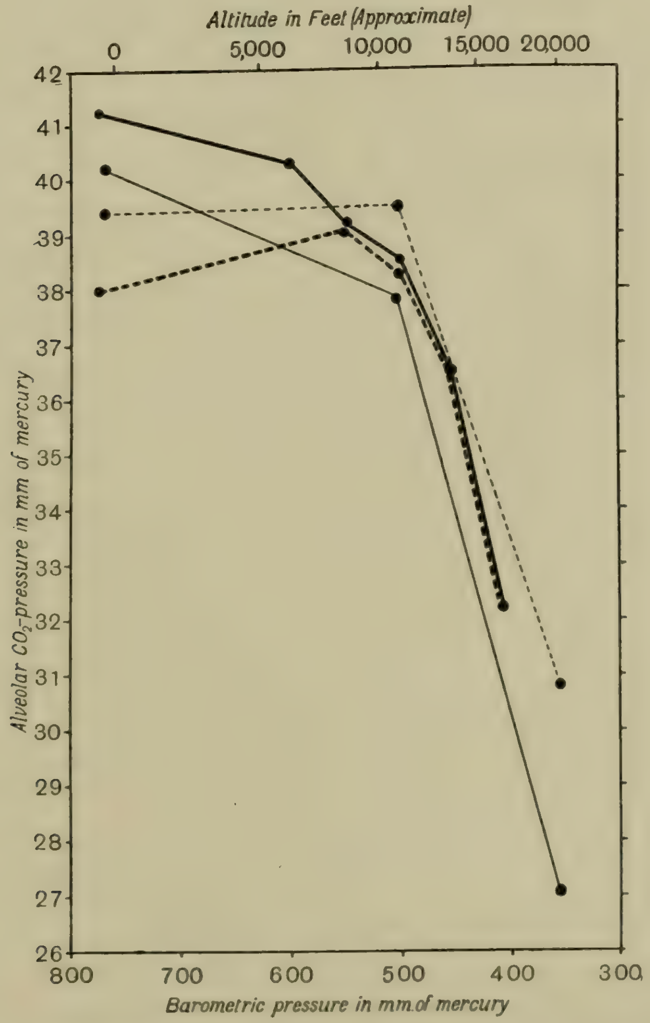

Fig. 1. The thick lines represent Exp. I., the thin lines Exp. II., the continuous lines the results for J. S. H., the interrupted lines those for A. E. B.

ordinary barometric pressure, or 60 % of the normal. Alveolar oxygen pressures are shown graphically in Figs. 3 and 4.

On comparing the results for J. S. H. and A. E. B., it will be seen that they differed somewhat. In J. S. H. there was already a diminution in alveolar CO_2 pressure at 550 mm. barometric pressure, while this was not the case in A. E. B. even at 550 mm. At the lowest barometric pressure (356 mm.) there was also a greater fall in alveolar CO_2 pressure in J. S. H. than A. E. B. The respiratory centre of J. S. H. thus seemed to answer better to the stimulus resulting from want of oxygen than that of A. E. B. In apparent correspondence with this fact A. E. B. suffered more from the effects of want of oxygen than J. S. H., and the observations at 356 mm. had, in fact, to be somewhat abruptly terminated owing to the symptoms of A. E. B., who became deeply cyanosed and seemed to be losing consciousness after a slight exertion due to a mistake with the gas analysis apparatus. As the pressure diminished below about 450 mm. both observers began to experience the usual symptoms of want of oxygen—more or less blueness of the face, difficulty in carrying out and calculating the analyses, loss of memory, etc. At 356 mm. these symptoms, and general discomfort, became very marked in A. E. B. The calculated alveolar oxygen pressure in the last observations at 356 mm. barometric pressure was 30·5 mm. in A. E. B. and 35·7 mm. in J. S. H., assuming the respiratory quotients to have been 0·9. The corresponding alveolar CO_2 pressures were 30·9 and 26·3 mm. The fact that the latter values are 25 and 33 % below the normal indicates that there was slight hyperpnœa, but this was not noticeable to the subjects of the experiment. What was noticed was that any slight holding of the breath, as in making observations, etc., was followed by discomfort and deep respirations.

A barometric pressure of 356 mm. corresponds to an altitude of only about 20,000 feet. This altitude has often been considerably exceeded by both mountaineers and balloonists without serious difficulty. The reasons why we, in common with other observers who have used steel chambers, were already in difficulties at 356 mm., will be discussed later.

Exps. III—VIII call for no special remarks, except that the alveolar oxygen pressure was also determined in many cases, and that although the mode and extent of decompression was varied a good deal the general results confirm those of Nos. I and II. Mr Ogier Ward and Dr Haldane suffered hardly at all from symptoms of lack of oxygen at 425 mm., while Dr Boycott experienced some discomfort at this pressure, but had no difficulty in making the analyses.

In Exp. IX the pressure was reduced to 400 mm. when all three observers had distinct symptoms of want of oxygen, accompanied by the expected fall of 4—6 mm. in the alveolar CO_2 pressure. About 100 cubic feet of oxygen were then discharged into the air from a large cylinder. This raised the pressure to 740 mm. The pump was then re-started, and the pressure reduced, first to 411 mm., and later to 307 mm. The oxygen percentage in the air was now about 45, and the alveolar oxygen pressure as high as 80 mm., even with the barometric pressure at 307 mm.

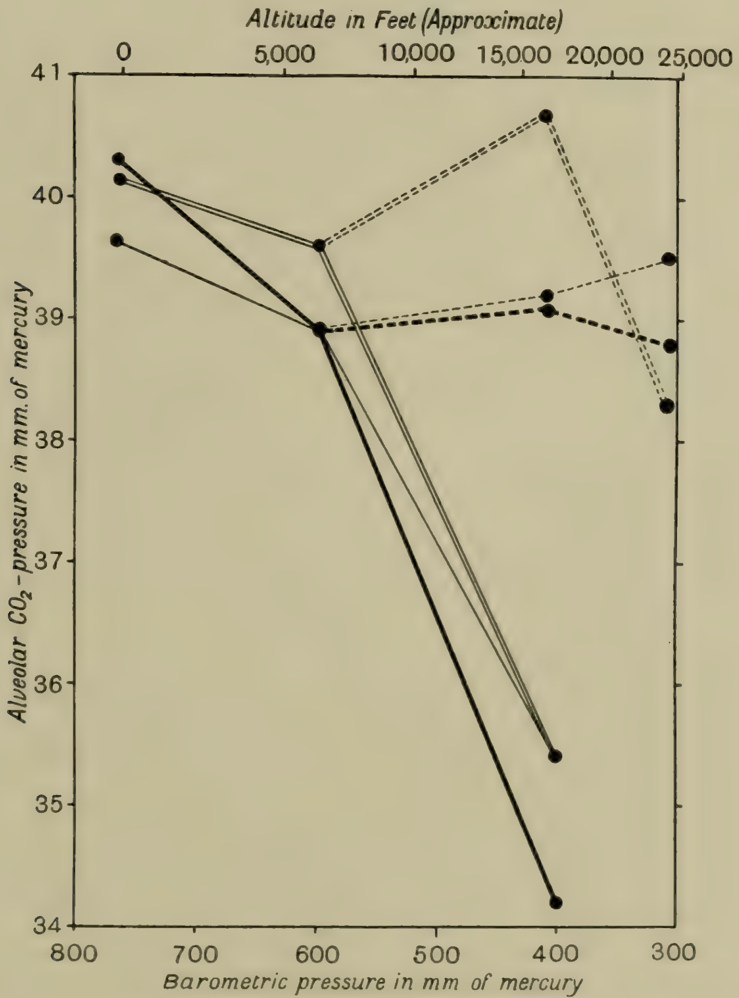

Fig. 2. The continuous lines represent the results in air, the dotted lines those in 45—50 % oxygen, the thick lines those for J. S. H., the thin lines those for A. E. B., and the double lines those for R. O. W.

The result was that none of us had the slightest symptoms of want of oxygen, and that the alveolar CO_2 pressure went back to normal, and remained practically normal, even at 307 mm. barometric pressure, although the chamber felt somewhat warm, as the ventilation was stopped[1].

This experiment shows clearly that the symptoms experienced, and the fall in alveolar CO_2 pressure in rarefied air, are alike dependent on want of oxygen and not on the mere diminution of pressure. The fall in alveolar CO_2 pressure, and consequent "acapnia," to use Mosso's

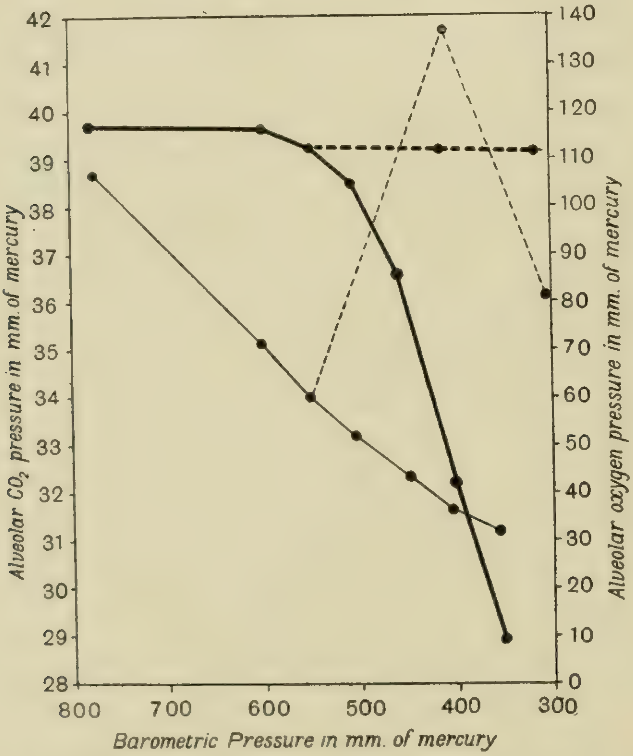

Fig. 3. Effects of diminished barometric pressure on the alveolar gas-pressures. The thick lines show the alveolar CO_2 pressure, and the thin lines the alveolar O_2 pressure. The dotted lines refer to the experiment in which oxygen was added to the air.

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[1] We may call attention at this point to what appears to be a risk in cases where the air-pressure is fairly rapidly lowered to points below about 300 mm. This can be done without risk of want of oxygen as was shown by Paul Bert, if arrangements are made for breathing oxygen, and Aggazzotti has recently succeeded, with the help of a mixture of oxygen and CO_2 in himself reaching the very low pressure of 122 mm. (corresponding to 48,000 feet), and of taking an ape to the pressure of 95 mm. without evident symptoms of oxygen-want being produced. Judging from the experimental data of Boycott, Haldane, and Damant, there would be increasing risk of disengagement of bubbles in the blood, and consequent symptoms of caisson disease, if the pressure were reduced too rapidly beyond about 300 mm. In the book quoted above (p. 121) von Schrötter records an attack of symptoms in himself resembling caisson disease when he was in a steel chamber, of which the pressure had fallen in 15 minutes to 230 mm. Although he concludes that these symptoms could not have been due to caisson disease, we think, in view of the data given by Damant and ourselves, that he was probably mistaken, and that the risk of caisson disease at very low pressures ought to be taken into account.

expression, are, as maintained by Zuntz and other supporters of Paul Bert's original theory, secondary to the want of oxygen, and not primary factors in the causation of mountain sickness and the other symptoms produced by low barometric pressure. Figs. 2 and 3 show graphically the changes in the alveolar gas-pressures in this experiment. In Fig. 3 are shown the variations in the average observed and calculated alveolar oxygen and CO_2 pressures of A. E. B. and J. S. H. in experiments I and II, and in dotted lines those observed after the oxygen had been liberated in the chamber in experiment VII. The effect of the oxygen in keeping the alveolar CO_2 pressure constant, in spite of the diminution in barometric pressure is clearly seen.

Fig. 4 (see also Appendix, p. 374) shows graphically the variations in A. E. B. of (1) the observed alveolar CO_2 pressure, (2) the observed alveolar CO_2 percentage (to which, of course, the deficiency in oxygen percentage runs parallel when air is breathed), and (3) the calculated or observed alveolar oxygen pressure. These values were obtained in

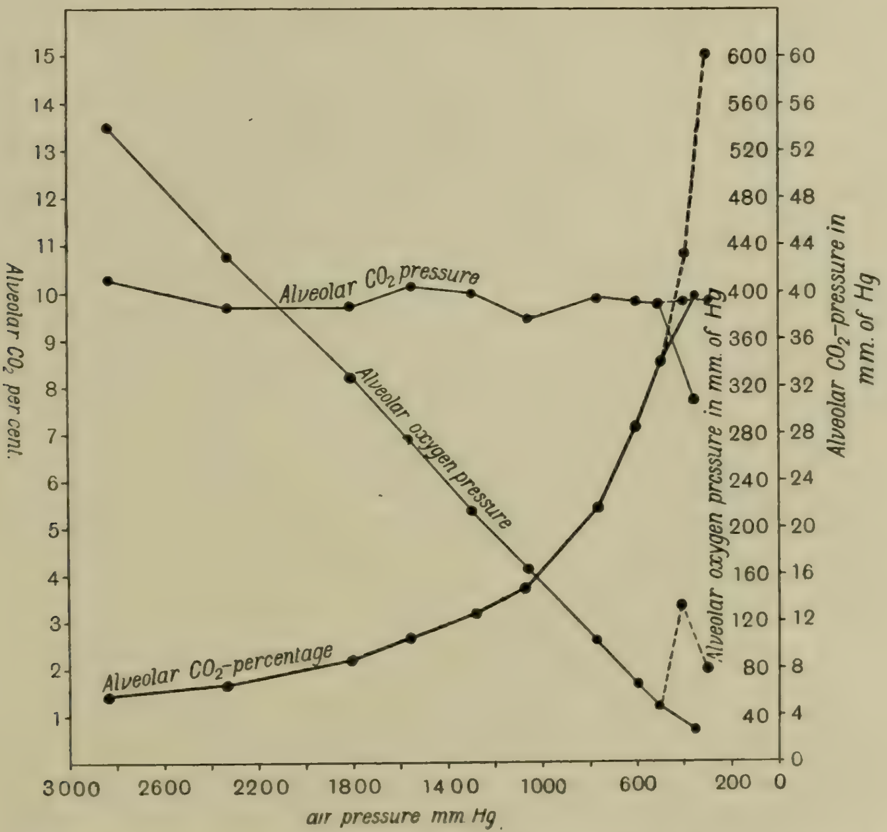

Fig. 4. Effects of variation in barometric pressure on alveolar gas-pressures and percentage of CO_2 in A. E. B. The dotted lines show results when oxygen was added to the air.

different experiments, at pressures varying from 2830 mm. (nearly four atmospheres) to 307 millimetres. The dotted lines indicate the results when oxygen was added to the air breathed. It will be seen that so long as the alveolar oxygen pressure exceeded about 50 mm. the alveolar CO_2 pressure remained practically constant, while the alveolar CO_2 percentage varied inversely as the barometric pressure (allowing for the aqueous vapour in the alveolar air). These observations furnish fresh evidence that the regulation of breathing, during rest, at any rate, is, within wide limits, independent of variations in the alveolar oxygen pressure.

In Exp. II (and to a less extent in Exps. I and IV), it was noticed in the case of J. S. H. that after exposure to a low air-pressure the alveolar CO_2 pressure did not return to normal for more than an hour after the pressure had been raised to normal. The determinations were repeated again and again during the interval after experiment II so that there could be no doubt of the fact. The exposure to want of oxygen seemed to have altered the normal standard of alveolar CO_2 pressure, and the alteration remained for some time after the want of oxygen had been entirely removed. This fact suggested that the fall of alveolar CO_2 pressure during exposure to want of oxygen is due in whole or part to some secondary change brought about by the want of oxygen, and not to an immediate effect of want of oxygen in exciting the respiratory centre and so producing hyperpnœa. This secondary change might evidently have a most important bearing on the adaptation of the body to low atmospheric pressures, as the alveolar oxygen pressure, other things being equal, must rise if a physiological change which diminishes the alveolar CO_2 pressure occurs. To investigate this and other matters more thoroughly Mr Ogier Ward undertook to make observations on himself during a stay of some days on Monte Rosa; and the results of his investigation are contained in the succeeding paper. He found that even at Zermatt (633 mm.) his alveolar CO_2 pressure became distinctly lower, remaining steady at the lower pressure, and that a stay on Monte Rosa (443 mm.) produced a much greater fall in alveolar CO_2 pressure than was observed at the same pressure in the chamber: also that this fall lasted on after his return to Zermatt. There could thus be no doubt that the slight hyperpnœa, and consequent fall in alveolar CO_2 pressure, produced at high altitudes, is due, not directly, but indirectly to want of oxygen; and that this indirect effect may continue for a considerable time after the lack of oxygen has been removed.

A further long experiment on the same subject was made in the steel chamber, with Mr Ogier Ward as the subject. The pressure was reduced to 540 mm., and he remained inside for 24 hours, with the pump running the whole time, so as to give ample ventilation. Fig. 5 shows graphically the results. It will be seen that although in the previous short experiments in the chamber a fall of barometric pressure to 545 mm. had only caused a fall of about 1 to 1·5 mm. in the alveolar CO_2 pressure, yet after 2 hours' exposure to this barometric pressure the alveolar CO_2 pressure had fallen 5 mm.; and about 7 mm. after 20 hours' exposure. It will also be seen that when the barometric pressure was again raised to normal the alveolar CO_2 pressure continued for about 20 hours to be nearly as low as during the experiment, and had scarcely returned to normal after two days[1].

It thus seems that the effect of long-continued scarcity of oxygen is to lower by some means the normal level of the alveolar CO_2 pressure. It is apparently still the alveolar CO_2 pressure that directly determines the lung ventilation. Want of oxygen is at best a very feeble direct stimulus to respiration, if it is a direct stimulus at all: for in the steel chamber cyanosis and loss of consciousness may be caused by want of oxygen, without any previous hyperpnœa to speak of. During a stay at high altitudes, as on Monte Rosa, muscular exertion produces unusually marked hyperpnœa. If this were due directly to want of oxygen we should expect to observe fainting with every exertion; but such fainting does not occur—only great hyperpnœa. This point was carefully observed by Ogier Ward on Monte Rosa. Extreme hyperpnœa is, of course, produced by rapidly diminishing the percentage of oxygen in the inspired air; and this phenomenon, the cause of which will be discussed in another paper in this *Journal* (p. 390), has led to the prevalent belief that want of oxygen is a direct stimulus to the respiratory centre.

As to the means by which the normal alveolar CO_2 pressure becomes lowered by exposure to want of oxygen, a number of facts suggest that diminished alkalinity of the blood is the actual cause. It is known that lack of oxygen leads to the formation of large quantities of lactic acid. This was clearly proved by a series of experiments carried out in Hoppe-Seyler's laboratory by Araki[2]. He found that as much as

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[1] In this experiment R. O. W. suffered from nausea after the first 12 hours, and slept badly in spite of a comfortable bed. The ventilation was sufficient to keep the CO_2 percentage below 0·15, and make the atmosphere feel fresh and cool, so that the nausea, etc. were doubtless due to the low pressure.

[2] *Zeitsch. f. physiol. Chem.* xv. p. 335. 1890; xvi. p. 425; xvii. p. 311; xviii. p. 422.

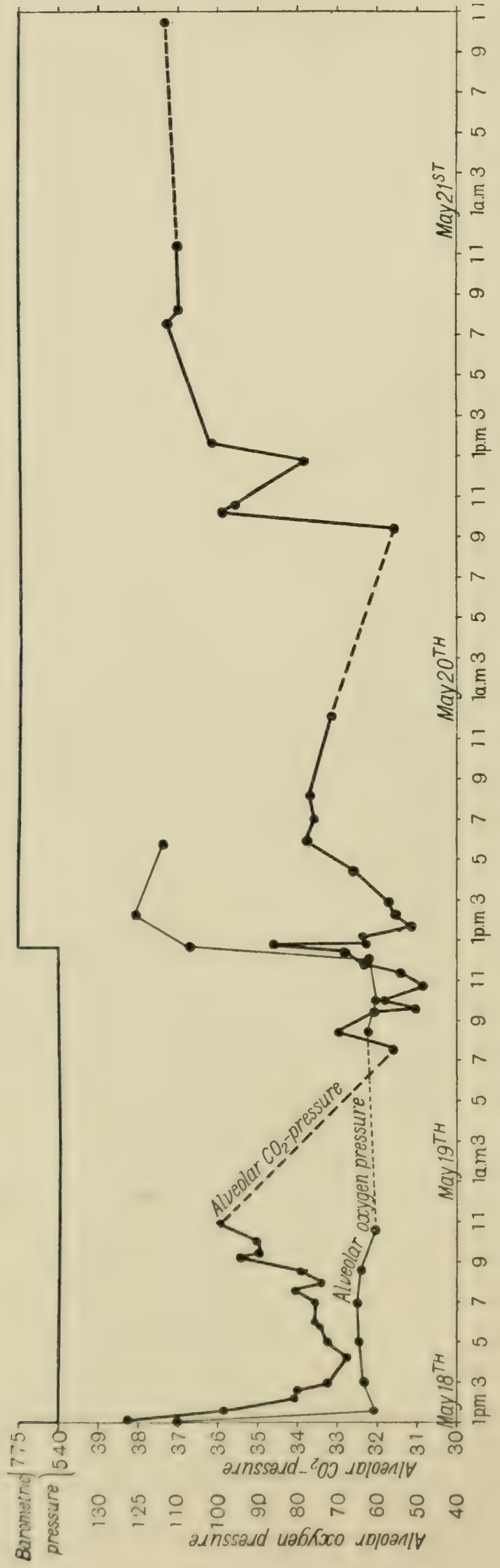

Fig. 5. Effects of 24 hours' exposure of R. O. W. to 540 mm. barometric pressure.

2 % of zinc lactate could be obtained from the urine of animals exposed to want of oxygen, and that not only was the amount of lactic acid in the blood greatly increased, but its alkalinity was very markedly diminished. Want of oxygen was produced by a low percentage of oxygen in the inspired air; by carbonic oxide poisoning, and by amyl nitrite poisoning. Any doubts as to the latter two substances producing want of oxygen, and causing death by want of oxygen, have now been removed[1]. Fletcher and Hopkins[2] have also recently demonstrated the influence of want of oxygen in the production of lactic acid in muscle; and finally Galeotti[3] and Aggazzotti[4] have found that the alkalinity of the blood was diminished on Monte Rosa and on exposure to low atmospheric pressure in a steel chamber.

It is known also that the ingestion of acids into the body causes hyperpnœa, accompanied by diminished alkalinity of the blood and a fall in the CO_2 percentage to even less than 3 %[5]; and that the injection of dilute sodium hydrate into the blood causes apnœa[6].

It seems reasonable, therefore, to conclude that the action on the respiratory centre of the circulating blood is due to what may be called its total acidity, including, of course, that due to free CO_2. If more lactic or other acid, or less alkali, is present in the blood than usual, then less free CO_2 will be required to excite the respiratory centre, and the alveolar CO_2 pressure will consequently fall; and *vice versa*. This theory seems to explain well the fall in alveolar CO_2 pressure at low barometric pressures. It also explains why this fall requires some time to develope, and does not disappear at once, since it will take some time for the body to develope the maximum percentage of lactic or other acid in the blood, or to recover from this condition. Probably also the presence of lactic acid in the blood leads to an elimination of lactate of soda and potash in the urine, and consequent diminution in the normal quantity of soluble alkali in the body, even although ammonia not converted in the usual manner into urea may also be eliminated in combination with lactic acid. Some such process as this seems probable in view of Bunge's work on the action of potash salts in robbing the body of sodium chloride; and if lactic acid robs the body of soluble alkali we have a ready explanation of the extreme slowness with which the alveolar CO_2 pressure returns to normal after prolonged exposure to low atmospheric pressure. Slight variations in the alkalinity of the blood may well explain the variations sometimes observed in the alveolar CO_2 pressure[1].

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[1] Haldane. This *Journal*, XVIII. p. 201. 1895. Haldane, Makgill and Mavrogordato. *Ibid.* XXI. p. 160. 1897.

[2] This *Journal*, XXXV. p. 247. 1907.

[3] *Arch. ital. de Biol.* XLI. p. 80. 1904.

[4] *Ibid.* XLIV. 1905.

[5] Walter. *Arch. f. exper. Pathol.* VII. p. 148. 1877.

[6] Hougardy. *Arch. internat. de Physiol.* I. p. 17. Mosso. *Arch. ital. de Biol.* XLII. p. 100. 1905.

The gradual fall in the alveolar CO_2 pressure at low atmospheric pressures is evidently a most important factor in explaining accommodation to low pressures; for of course a fall in alveolar CO_2 pressure implies a rise in alveolar oxygen pressure, other things being equal. The importance of this factor will be evident from a single example. Let us suppose that the barometric pressure is reduced to half (i.e. to 380 mm.), and that the alveolar CO_2 pressure remains normal at 40 mm., with a respiratory quotient of ·9. The alveolar oxygen pressure will evidently be $40 \times \frac{10}{9} = 44{\cdot}4$ mm. below the oxygen pressure of the inspired air, allowing for the tension of aqueous vapour in alveolar air (46 mm.). But the total oxygen pressure of the inspired air saturated at the body temperature is $(380 - 46) \times \frac{20{\cdot}9}{100} = 69{\cdot}8$ mm., so that the oxygen pressure of the alveolar air would only be $69{\cdot}8 - 44{\cdot}4 = 25{\cdot}4$ mm. This would cause extreme cyanosis, and probable loss of consciousness, to judge from all our experience. If, on the other hand, the alveolar CO_2 pressure were regulated to about 25 mm., as would probably be the case, judging from Mr Ogier Ward's observations on Monte Rosa, the oxygen pressure in the alveolar air would be $69{\cdot}8 - 27{\cdot}8 = 42$ mm.—a value at which little or no discomfort would be experienced, to judge from our experience. In the short experiment where A. E. B. became ill at a pressure of 356 mm. his alveolar CO_2 pressure had only had time to fall to 31 mm., so that his alveolar oxygen pressure was only about 30·8 mm., which seems to be about the limit where serious symptoms begin.

If the foregoing conclusions are correct it would seem to be a matter of great importance to mountaineers and balloonists to keep the respiratory quotient as high as possible: for the higher the respiratory quotient the higher will the alveolar oxygen pressure be, other things being equal. A mainly carbohydrate diet, and avoidance of long intervals between meals, would therefore seem advisable. The breathing of air to which CO_2 has been added without at the same time abstracting oxygen would, of course, have a similar effect, as has been actually proved by Mosso and his pupils. Nothing in our experiments, however, lends any support to Mosso's theory that the primary cause of mountain-sickness is lack of CO_2 ("acapnia"). The relief produced by excess of CO_2 is due, at least mainly, to the artificial production of an abnormal hyperpnœa, and consequent rise in the alveolar CO_2 pressure. The condition of the body then becomes one of "hypercapnia"; and indeed "hypercapnia" may be said to be already present at high altitudes, without the addition of CO_2 to the inspired air, since the stimuli to the respiratory centre are already in excess of the normal.

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[1] Beddard, Pembrey and Spriggs have recently shown that during diabetic coma, which is associated with greatly diminished alkalinity of the blood, the alveolar CO_2 pressure may fall to as low as 8·3 mm. (This *Journal*, XXXVII. p. xl. 1908.)

Our conclusions as to respiration under low pressure seem to harmonise well with the important and clearly thought-out observations of Zuntz and his associates. They have shown that the volume of air breathed at high altitudes is increased, as a rule, out of proportion to any increase in the rate of respiratory exchange, so that the alveolar oxygen pressure is higher, and the alveolar CO_2 pressure lower, than would otherwise be the case. They also attribute this increase to the formation of acid substances as a consequence of insufficient oxygen supply to the body, but appear to regard the stimulus of these acid substances as wholly replacing that of CO_2 at very high altitudes, as, for instance, on Monte Rosa[1]. That such acid substances could be formed or could disappear sufficiently promptly to take the place of CO_2 as an efficient regulator of the lung ventilation seems very improbable, however, in view of our observations; and we think that the theory we have put forward explains more satisfactorily the facts relating to respiration at low pressures. More direct evidence of the paramount importance of CO_2 in regulating the breathing, whether or not want of oxygen is present, will be furnished in a further paper by Mr E. P. Poulton and one of us.

The adaptation to low air-pressures which we have investigated is only one of, in all probability, several. There is also the well-known gradual increase in the percentage of hæmoglobin; but besides this there is almost certainly an adaptation of the circulation. As was recently shown by Miss FitzGerald at a meeting of the Pathological

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[1] Zuntz, Loewy, Müller, and Caspari, *loc. cit.* pp. 443, 444. Zuntz and Geppert have put forward a similar theory of the hyperpnœa due to muscular exertion. The objections to this theory have already been stated by Haldane and Priestley (this *Journal*, XXXII. p. 262. 1905). We think, however, that where muscular work is excessive, so that partial muscular asphyxia occurs, the lactic acid formed contributes to the stimulus to the respiratory centre.

Society of Great Britain, the alveolar CO_2 pressure is practically normal during rest in cases of chlorosis, where the oxygen percentage in the blood is often reduced to a third or less—far lower than at the barometric pressures which we have investigated. This can only be explained on the theory that the blood-flow is sufficiently increased in chlorosis to compensate for the deficiency of oxygen in the blood. This adaptation must occur slowly, since a sudden reduction of 70 °/₀ in the oxygen-carrying power of the blood, as in CO-poisoning, causes unconsciousness and probable death. That a similar circulatory adaptation occurs gradually at high altitudes seems extremely probable; but neither this adaptation nor the increase of hæmoglobin could raise to more than a limited extent the oxygen pressure in the blood passing through the tissues, so that respiration could hardly be expected to become normal again during rest, as it does in chlorosis. The activity of the lung epithelium in absorbing oxygen may also be gradually increased, but even this could not be expected to bring back the respirations to normal with pressures of less than 300 mm.

In conclusion, we wish to express our obligation to the Director of the Lister Institute for the facilities which he placed at our disposal, and to Dr Rowland of the Lister Institute and Mr Ogier Ward for the help which they gave us in several of the experiments.

Part of the expense of apparatus was defrayed by a grant from the Scientific Grants Committee of the Royal Society to one of us.

SUMMARY OF CONCLUSIONS.

1. When the atmospheric pressure is diminished, the alveolar CO_2 pressure remains constant during short exposures in accordance with the law of Haldane and Priestley, till the air-pressure falls to about 550 mm., or the alveolar oxygen pressure to about 62 mm., but at lower air-pressures falls with increasing rapidity.

2. With long exposures the alveolar CO_2 pressure begins to fall at a less low atmospheric pressure, and at any sufficiently low pressure falls considerably further than with short exposures, the alveolar oxygen pressure being thus rendered correspondingly higher, so that the process is one of gradual adaptation to low pressures or great altitudes.

3. The fall in alveolar CO_2 pressure is due to hyperpnœa dependent on the low alveolar oxygen pressure, and is entirely absent if oxygen is

added to the air, so as to prevent excessive fall of alveolar oxygen pressure.

4. This hyperpnœa is not immediately due to want of oxygen, since it takes some time to develope, and may remain for days after the want of oxygen has been removed. It is probably due to the gradual formation, in consequence of insufficient oxygen supply, of lactic acid or other substances which have the same influence as CO_2 on the respiratory centre, so that less CO_2 is required to excite the centre.

5. When the alveolar oxygen pressure falls to about 30 mm. urgent symptoms (marked cyanosis and tendency to fainting) are produced.

6. At both high and low atmospheric pressures the breathing is so regulated as to maintain a certain pressure of CO_2 in the alveolar air; but as this pressure is lower at very low barometric pressure than at normal or high barometric pressures, the lung ventilation is increased at low barometric pressures, and excessive fall in the alveolar oxygen pressure is thus prevented.

7. Exposure to cold causes a slight rise, and to heat a slight fall, in the alveolar CO_2 pressure.

APPENDIX.

Experiment I.

			Alveolar air			
			J. S. H. CO_2		A. E. B. CO_2	
Time	Pressure	Temp.	per cent.	mm.Hg	per cent.	mm.Hg
1.30	774	16·5°	5·72	41·6	5·32	38·7
			5·60	40·8	5·00	36·4
					5·33	38·8
2.56	601	17·5°	7·29	40·4	6·98	38·7
			7·22	40·1	7·01	38·9
3.34	549	17·5°	7·72	38·8	7·67	38·6
			7·90	39·7	7·90	39·7
					7·73	38·9
4.19	502	18·3°	8·60	39·2	8·42	38·4
			8·44	38·5	8·30	37·8
			8·38	38·1	8·48	38·7
5.20	457	18·7°	8·78	36·1	9·09	37·4
			8·95	36·8	8·64	35·5
					8·84	36·3
5.58	407	18·9°	8·91	32·1	8·98	32·4
			8·94	32·3	9·04	32·6
6.38	774	18·6°	5·05	36·8	5·47	39·8
			5·18	37·7	5·47	39·8
			5·47	39·8	5·43	39·5
					5·58	40·6
					5·36	39·0

Experiment II.

			Alveolar air			
			J. S. H. CO_2		A. E. B. CO_2	
Time	Pressure	Temp.	per cent.	mm.Hg	per cent.	mm.Hg
3.0	770	17°	5·54	40·1	5·47	39·6
			5·56	40·2	5·57	40·3
					5·29	38·3
3.43	501	19°	8·07	36·7	8·35	38·0
			8·40	38·2	8·61	39·2
			8·51	38·7	8·69	39·5
			8·35	38·0	8·84	40·2
					8·74	39·8
					8·90	40·5
5.4	356	19·7°	8·98	27·8	10·13	31·4
			8·50	26·3	9·96	30·9
5.53	772		4·78	34·7	5·47	39·7
			5·14	37·3	5·22	37·9
			5·09	36·9	5·53	40·1
			5·27	38·3		
			5·20	37·7		
			5·52	40·1		

Experiment III.

			Alveolar air									
			J. S. H.		A. E. B.				R. O. W.			
			CO_2		CO_2		O_2		CO_2		O_2	
Time	Pressure	Temp.	per cent.	mm.Hg	per cent.	mm. Hg	per cent.	mm. Hg	per cent.	mm. Hg	per cent.	mm. Hg
3.30	773	21°							5·21	37·9		
3.43	642	22°	6·50	38·7	6·69	39·9			6·53	38·8	14·86	87·6
4.16	541	22°	7·53	37·3	7·90	39·1			7·37	36·4	11·21	55·5
4.47	425	22·5°	8·85	33·8	9·72	36·9			9·36	35·3	10·26	38·9
5.25	773	23°	5·14	37·4	5·63	40·9			5·10	37·2		

Experiment IV.

1.30	774	16°			5·43	39·4	14·82	107·9	5·18	37·6	15·61	113·6
					5·60	40·8			5·22	37·9	15·26	111·1
					5·67	41·2						
2.42	640	17·5°			6·94	41·2			6·19	36·6	15·17	90·1
					6·79	40·3			6·32	37·5	14·00	83·2
					6·81	40·4						
					6·80	40·4	13·34	79·2				
3.40	538	19°	7·88	38·9	8·42	41·4			7·35	36·4	12·58	61·9
					8·48	41·8	12·28	60·4	7·26	35·9	12·50	61·5
4.21	458	19·5°	8·50	35·0	9·71	40·0			8·64	35·6	11·35	46·7
					9·03	37·2	10·99	45·3	8·69	35·8		
					9·12	37·6	10·98	45·2				
					9·30	38·3						
5.5	774	18°	5·62	40·8					5·08	36·8		
									4·97	36·0		

Experiment V.

			Alveolar air					
			J. S. H. CO_2		A. E. B. CO_2		R. O. W. CO_2	
Time	Pressure	Temp.	per cent.	mm. Hg	per cent.	mm. Hg	per cent.	mm. Hg
1.30	763	18°	5·69	40·8	5·14	36·8	5·41	38·8
					5·38	38·6	5·54	39·7
					5·35	38·4		
3.0	563	20°	7·09	39·7	7·71	39·9	7·64	39·5
					7·30	37·7	7·64	39·5
3.40	425	20°	9·43	35·7	9·67	36·6	9·79	37·3
					9·42	35·7	9·69	36·9
4.14	763	21°	5·32	38·1	5·50	39·4	5·59	40·0
							5·61	40·2

Experiment VI.

			Alveolar air							
			A. E. B.				R. O. W.			
			CO$_2$		O$_2$		CO$_2$		O$_2$	
Time	Pressure	Temp.	per cent.	mm. Hg	per cent.	mm. Hg	per cent.	mm. Hg	per cent.	mm. Hg
2.0	769	18°	5·73	41·4	14·29	103·3	5·16	37·3		
			5·79	41·9	14·85	107·4	5·00	36·1		
			5·85	42·3	14·65	106·0				
3.25	620	19°	6·93	39·8	13·45	77·2	6·39	36·6	13·07	75·0
			6·81	39·1	13·46	77·3	6·45	37·1	13·24	76·0
							6·52	37·5	13·50	77·5
4.40	495	20°	8·77	39·4	11·68	52·4	8·02	36·0	11·46	51·5
			8·83	39·6	11·01	49·4	7·99	35·9	11·98	53·8
5.28	768	20·5°	5·60	40·4			5·35	38·6		
			5·62	40·6			5·28	38·1		

Experiment VII.

			Alveolar air. A. E. B.	
Time	Pressure	Temp.	CO$_2$ per cent.	CO$_2$ mm. Hg (mean)
1.30	761	18·7—19·0	5·66, 5·74, 5·45	40·2
2.27	494	18·0—19·8	8·59, 8·51, 8·43	38·1
2.52	549	20·6—19·6	7·70, 7·18, 7·56	37·6
3.15	597	20·8—20·0	7·01, 7·17, 6·80	38·5
3.37	648	21·0—20·4	6·79, 6·39, 6·55	39·6
4.0	697	21·4—20·2	5·93, 5·90	38·5
4.16	761	21·8—20·8	5·65, 5·38	39·4

Experiment VIII.

			Alveolar air. A. E. B.	
Time	Pressure	Temp.	CO$_2$ per cent.	CO$_2$ mm. Hg (mean)
2.11	771	17·4—18·4	5·57, 5·41	39·8
2.42	470	16·2—18·4	9·28, 9·06	38·9
2.58	526	20·0—18·8	8·44, 8·33	40·3
3.12	573	20·0—19·0	7·80, 7·50	40·3
3.27	621	20·4—19·2	7·28, 7·01	41·1
3.45	672	20·4—20·5	6·55, 6·28	40·1
4·3	770	21·2—19·8	5·60, 5·69	40·9

Observations of Alveolar CO$_2$ in different experiments under high atmospheric pressure (A. E. B.).

Pressure mm. Hg.	CO$_2$ per cent.	=	mm. Hg.
2830	1·49		41·4
2330	1·70		38·8
1810	2·21		39·0
1550	2·70		40·6
1290	3·22		40·1
1035	3·83		37·8
763	5·51		39·5

Experiment IX.

					Alveolar air											
			Air		J. S. H.				A. E. B.				R. O. W.			
Time	Pressure mm. Hg	Temp. cent.	CO_2 p.c.	O_2 p.c.	CO_2 p.c.	= mm. Hg	O_2 p.c.	= mm. Hg	CO_2 p.c.	= mm. Hg	O_2 p.c.	= mm. Hg	CO_2 p.c.	= mm. Hg	O_2 p.c.	= mm. Hg
2.0	763	18°		20·9	5·62	40·3			5·58	40·0	14·48	103·8	5·55	39·8		
									5·48	39·3	14·89	106·8	5·65	40·5		
3.10	596	19°			7·07	38·9			7·03	38·7			7·15	39·3		
									7·10	39·0	[12·7	= 69·8]	7·27	39·9		
3.40	400	19°	0·43	20·64	9·65	34·2			10·02	35·5			10·08	35·5		
									10·01	35·4	[9·6	= 34·0]	9·99	35·3		
4.25	740	19°														
4.34	411	19°	0·54	50·03	10·72	39·1			10·51	38·4			11·18	40·8		
									10·95	40·0	37·76	137·8	11·09	40·5		
5.5	307	20°	2·58	45·20	15·37	39·6			15·64	41·3			15·22	38·6		
					14·57	38·0	32·03	84·3	14·52	38·3			14·85	39·0		
									14·91	38·9	30·84	80·5	14·35	37·4	31·08	81·1
6.0	763	20°		20·9					5·79	41·5			5·81	41·6		

ON THE ORIGIN OF ENDOGENOUS URIC ACID

BY

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ON THE ORIGIN OF ENDOGENOUS URIC ACID

By E. P. CATHCART, E. L. KENNAWAY and J. B. LEATHES

In a recent paper by Hirschstein, the fact that the rate at which uric acid is discharged from the body is greater during the day than it is at night is explained as being due to the retention of uric acid during sleep, and the fact that it is during the first waking hours that this rate is greater than at any other time in the day is ascribed to the rapid discharge of the uric acid that has accumulated during the night. This theory, which seems to imply that the rate at which the uric acid is produced in the body is fairly constant at all times of the day, is supported by the following experimental evidence :—

After a day on which food free from purin derivatives was given, a patient was put on a regular diet of three meals at 7 a.m., noon, and 6 p.m., each of which had the same composition, bread, milk, butter, an egg and an apple. On the third day, in addition to the usual breakfast, 250 g. of calf's thymus was given, on the fifth day the same addition was made to the midday meal, and on the seventh day to the evening meal. The considerable increase in the output of uric acid, that is known to follow such an amount of thymus as this, might be expected, if there is a tendency for uric acid to be retained at night, to appear in the urine later when the thymus was given in the evening, than when it was given in the morning. The urine was collected and examined every eight hours, but the results may be given as follows :—

Table I.

	Mg. uric acid excreted		Average in 24 hours
	24 hours	48 hours	
1st day	284	585	292
2nd day	301		
3rd day + 250 g. thymus at 7 a.m. .	385	612	306
4th day	227		
5th day + 250 g. thymus at 12 noon	301	469	234
6th day	168		
7th day + 250 g. thymus at 6 p.m. .	261	385	269
8th day	124		
9th day	423		

In the last column but one of this table the output of uric acid in successive periods of 48 hours each is given, in the first of which periods no thymus was given, in the next three, at some time in the course of the first 12 hours out of the 48, 250 g. of thymus was added to the ordinary food. If the output

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on the first two days is taken as a standard for comparison, as is presumably intended, then we have to note the remarkable fact, that the addition of the thymus caused an increased output of uric acid amounting to 30 mg. on the first occasion on which it was given, but after that the subsequent additions of thymus resulted actually in a smaller, and in fact considerably smaller, output than on the days when the food contained no purin compounds. Now Burian and Schur reckon that 100 g. of calf's thymus contains 0·45 g. of nitrogen in the form of purin bases; 250 g. therefore contain 1·125 g. Of this they find about one quarter or 0·28 g. appears in the urine, which, if it were all in the form of uric acid, would amount to 0·84 g., or allowing for some less completely oxidized purin base, to anything from 0·5 to 0·75 g. of uric acid. The figures given by Hirschstein are of quite a different character, and so far from justifying inferences as to the normal course of the excretion of endogenous uric acid, seem rather to call for some explanation of their abnormality. Whether that might be found in the fact that the subject selected for the experiment was convalescent from pneumonia and not yet out of bed, it is not possible to say. But during and after the crisis of pneumonia a great loss of nuclear material is known to occur, and it is possible that this loss may during convalescence be followed by retention of purin compounds in the food.

Before the appearance of Hirschstein's paper, one of us had tried to meet the possibility of the low output of uric acid at night being ascribed to retention, in some experiments to be described later. But the indications given by these experiments seemed to point to a conclusion different from that for which Hirschstein argued. And it therefore seemed necessary to repeat the experiment with the thymus under conditions which might be expected to lead to a less confusing result.

From September 1 to September 29 (vide protocols at end of this paper. Table IX) four meals were taken daily at 8.30 a.m., 12.30, 4.30 and 8.30 p.m., each consisting as in earlier experiments of a pint (570 c.c.) of milk, 6 ozs. (170 g.) of bread and 1 oz. (28 g.) of butter, and three preserved plums. The urine was collected at these hours immediately before the meals, and again at 11.30 p.m.: the five samples being analysed separately. The average rate at which uric acid was excreted at each of these periods of the day was calculated from the actual observations extending over nearly four weeks, not counting those periods in which the effect of the thymus taken could be detected. Calf's thymus, 173 g., was taken on two occasions, at 8.30 a.m. on the 13th and at 8.30 p.m. on the 14th, it being substituted for the amount of bread calculated to have a corresponding food value.

In the following table the effect on the output of uric acid is shown, the figures given being the amount excreted over and above the average amounts for the corresponding times of the day :—

TABLE II.

Amount of additional uric acid excreted over and above the normal average was	After taking 173 g. thymus at 8.30 a.m.	After taking 173 g. thymus at 8.30 p.m.
In the first 12 hours	204	221
In the first 24 hours	410	444
In the first 28 hours	428	476

After 28 hours in each case the observed rates were normal.

The curves in Fig. 1 show the rate at which this additional uric acid was excreted in each of the four-hour periods—the dotted line, that after the thymus had been taken in the morning; the unbroken line after it had been taken in the evening. The horizontal abscissa marked out into periods of four hours each, represents in each of these periods the average normal rate of excretion of uric acid for that period.

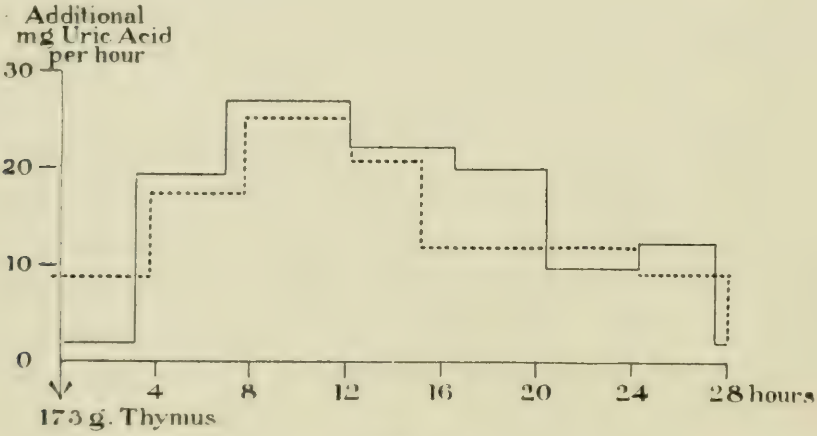

FIG. 1.

This figure shows clearly that there was not the slightest indication of retention of uric acid at night, when the thymus was given in the evening. The amount of uric acid excreted over and above the average amount after each meal of thymus agrees fairly well with the estimations of Burian and Schur. In 173 g. of thymus, containing 0·45 % of nitrogen in the form of purin bases, there would be 0·778 g. of such nitrogen. Of this, one quarter should appear in the urine, or 0·195 g. if the total purin compounds excreted were determined. The uric acid alone was estimated, and in 0·428 and 0·476 g. of uric acid there is 0·143 and 0·159 g. of nitrogen respectively. Had the total purin bases been determined, these figures would have risen to values very close to the normal estimates of Burian and Schur.

This result therefore is confirmed by its consistency with previous experience, and if it is fair to argue from the fate of exogenous to that of endogenous uric acid, which is the premiss on which Hirschstein's argument is based and probably correctly based, then the inference is that the output of uric acid is normally low at night and high in the morning, not because as much uric acid is formed during the night as during the day and some of it retained till the morning, but because the rate of production is lower during the night than the day, and higher in the first hours of the day than during the later hours. From this too it follows, as was argued in an earlier paper (Leathes), that the diurnal fluctuations in the rate of uric acid excretion correspond to fluctuations in the activity of some function or functions of which the endogenous uric acid is the chemical expression.

It is possible to induce diuresis and an increase in the rate of excretion of nitrogen, to overcome, as it might be put, a tendency to the retention of nitrogen at night, by drinking water at intervals during the night. This was done on Feb. 23, 1907 (Table VI), when at 1 a.m. and again at 5 a.m. 200 c.c. of water were taken. The nitrogen output and also that of creatinin was above the average in both the next samples of urine, collected at 8.30 a.m. and 2 p.m., but the output of uric acid was, notwithstanding, below the average in both periods. An attempt was also made to test this point, on the lines of the better experiment devised by Hirschstein and published some months later, by taking urate of sodium in the evening on Feb. 26, and in the morning of Feb. 28. But the fact that uric acid is very slightly absorbed from the intestine had been overlooked, and no definite result was attained.

Hirschstein however, having stated that the daily fluctuation in the output of endogenous uric acid is undoubtedly due to nocturnal retention, and that his experiment proves it, nevertheless in another part of his paper raises the question whether it may not be due to other causes, muscular activity on the one hand or activity of the digestive organs on the other. That muscular activity does not influence the output he considers is proved by an experiment in which a patient taking the same purin-free food on four successive days, excreted on the first two days when he was kept in bed, 0·381 and 0·359 g. of uric acid, and then on the next two days, when he was up, excreted 0·300 and 0·332 g. But as a result of his experiments on the relation of activity of the digestive organs to endogenous uric acid he concludes that at least 70 % of the output is to be attributed to this form of activity, a conclusion which there must be difficulty in reconciling with his conclusion as to the significance of nocturnal retention which we have been discussing.

In an earlier paper (Cathcart and Leathes) an experiment was described in which it was shown that during severe muscular exertions the output of uric acid was diminished, but that later, after the activity had ceased, an increase in the rate of output occurred which lasted for one or two days. An epitome of the evidence which we have collected on these points is here given, and shows, since most of it was obtained from other subjects and under varying conditions, that they may be generally applied.

1. Aug. 20, 1906. (J. B. L.) 8th day of bread, butter, and milk diet, taken in identical meals every 3 hours from 7 a.m. till 10 p.m. The urine was examined every 3 hours. Between 1 and 4 p.m. the subject bicycled for 2½ hours

on a hilly road, about 30 miles in hot weather. The rate of uric acid excretion during the 3 hours was 13 mg. per hour, as compared with the average rate for that time of day, 30·7 mg. per hour, and the rate during the preceding 3 hours 35·9 mg. per hour. During the following 24 hours the total output was 0·640 g. and during the next 24 hours after that 0·568 g. The total nitrogen excretion during the ride was at the rate of 0·83 g. per hour, and the average rate for this time of day 0·81 g. per hour.

2. May 28 and 31, 1907. (E.P.C., vide protocols, Table VIII.) On the first of these occasions muscular exertions were carried out stripped, and heat loss was accelerated by cold sponging. In the hours during which the muscular work was done, the rate of uric acid excretion fell from the average rate for that period of the day of 13 mg. to 6·2 mg. per hour: nevertheless, in the 24 hours beginning with the exercise the total output amounted to 0·396 g. as compared with the average of 0·321 g. As is pointed out in the paper where the experiment is fully described, this increased output is in part due to the effect of cold. Three days later severe exertions were again undertaken, only this time in heavy clothing. The output for the period during which the muscular activity occurred was at the very low rate of 3·6 mg. per hour as compared with the average rate of 13 mg. For some time after the rates were low, so that in the 24 hours beginning from the period of activity only 0·223 g. was excreted: in the two succeeding periods of 24 hours, however, the output was above the average—0·375 and 0·354 g. respectively—the average output for 24 hours being 0·325 g.

3. Sept. 10, 1907. (J. B. L., v. Table IX.) In the course of 3 hours two 10 lb. dumb-bells were lifted 720 times from the ground to above the head, and other active movements, running and jumping, were carried out in the ntervals. The rectal temperature, which was 99·0° at the beginning, rose to 101·2° and was over 100° during the greater part of the time. The uric acid output during the 4-hour period in which the exercise was taken fell from the average rate for this period of 30·4 to 19·9 mg. per hour, but rose in the next period to 44·6 (average 30·6) and was maintained above the average for 48 hours. In the first 24 hours after the exertion the output was 0·866 g., in the second, 0·715: the average for 24 hours for the whole experiment (22 days) was 0·652. In this case the total nitrogen excretion was 26 % below the average during the exercise, and during the first 24 hours the output of nitrogen was 2·7 g. below the average (15·6 g. instead of 18·3). The subjective effect was a great amount of general stiffness and soreness, especially in the back and shoulders.

4. Sept. 19, 1907. (J. B. L., v. Table IX.) At the same time of the day and for the same length of time, similar movements were carried out, only the weight of each dumb-bell was 3 lbs. instead of 10. The exertions involved therefore considerably less work, and were also carried out with considerably greater ease, owing to the practice acquired in the previous experiment. They were accordingly followed by far less fatigue and stiffness. The output of uric acid in the period including the exercise was at the rate of 23·5 mg. as

compared with the average for this period of 30·4, and with the figure on Sept. 10 of 19·9 mg. per hour. But in the succeeding 24 hours the output was just about the normal average 0·644 g. (average 0·652). The total nitrogen excretion was hardly affected at all. The temperature during the work did not rise to 100° at any time.

5. Sept. 28, 1907. (J. B. L., v. Table IX.) During the whole of this month the meals of bread, butter and milk were taken at 8.30 a.m., 12.30, 4.30 and 8.30 p.m. On this day the first meal was omitted, but one pint of water was taken instead of the usual pint of milk. The exercise, bicycling, began at 10, and at 12.30, when 23 miles had been covered, the first meal was taken. About 50 miles were completed by 4.30. The riding was against a strong wind, and there were delays due to the meal at 12.30 and later to accidents. The output of uric acid from 8.30 to 12.30 was at the rate of 29·3 mg. per hour (the average rate for these hours = 31·9), from 12·30 to 4.30 34·8 mg. per hour (average rate 30·4), while in the period from 4.30 to 8·30 after the ride was over, it rose to 47 mg. per hour when the normal average rate was 30·6. In the 24 hours ending at 12.30 p.m. on the 29th 0·806 g. of uric acid was excreted, the average for 24 hours in this month being 0·652 g. The nitrogen excretion was low during the ride, partly due to the fact that the first meal of the day had been omitted, but in the 24 hours ending at 12.30 on the 29th, the total output was about 2 g. above the average.

6. Nov. 7, 1907. (E. L. K., v. Table X.) This and the next two experiments were part of a long series, for which the subject took daily, from October 30 to December 1, three identical meals of bread and milk at 9 a.m., 2 and 7 p.m. The urine was collected at these hours, and also at 12 at night. On November 7, four hours' moderate exercise was taken, walking, from 10 a.m. to 2 p.m. The rate of uric acid excretion was during this period slightly higher than it had been at this period of the day. But during the night and on the following day it was noticeably increased, 0·483 g. being excreted, as compared with the average from 21 normal days, of 0·429.

7. Nov. 9, 1907. (E. L. K., v. Table X.) Severe muscular work, sawing and chopping wood, was done from 10 a.m. to 3 p.m. The uric acid output from 9 a.m. to 2 p.m. was at the rate of 19·3 mg. per hour, being somewhat below the average rate on normal days, which was 23·3 mg. per hour. From 2 p.m. to 7 p.m. it was at the rate of 50·2 mg. per hour, the normal rate being 20·7. In the 24 hours ending at 9 a.m. on the 10th 0·796 g., in the next 24 hours 0·648 g. and even in the third 24 hours 0·471 g. were excreted, the normal average amount for 24 hours being 0·429 g. In the three days, therefore, after the beginning of the work 0·628 g. more uric acid was excreted than was normal. The exertions were such as to cause intense stiffness and aching all over the body, lasting for several days.

8. Dec. 1, 1907. (E. L. K., v. Table X.) On this day the meal at 8.30 was omitted, but an amount of water was taken equal to the amount of milk that was usually taken. From 9 a.m. to 11 a.m. severe dumb-bell exercises were

performed. (10 lb. in each hand lifted 1,000 times from the ground to above the head). The urine excreted from 9 to 11 a.m. was analysed separately from that from 11 to 2 p.m. In the former, the uric acid corresponded to an output of 4·8 mg. per hour, in the latter to 32 mg. per hour, the average normal rate from 9 a.m. to 2 p.m. being 23·3 mg. per hour. From 2 to 7 p.m., when the experiment ended, the rate rose further to 48 mg. per hour, the normal being 20·7.

From the results of these experiments the two facts which stand out clearly are—

(1) That *during severe muscular exertions the output of uric acid is lowered*; the output of nitrogen in all forms is generally unaffected, and that of creatinin is also only slightly if at all altered (vide protocols at end of the paper). There were only two occasions on which the uric acid was not diminished. In Experiment 6 of the above list, when the exercise consisted merely of walking for 4 hours, the rate of excretion during the walk was slightly greater than it had been before at this time of day, and during the latter part of the bicycling in Experiment 5 it was a little above the average: but then, about 1½ hours out of the 4 hours of this period were spent resting for food and for repairs to the bicycle.

(2) *Muscular activity is followed by the excretion of uric acid in greater amounts than are normal, and this increased excretion may last for* 48 *or* 72 *hours.* If the exertions are considerable, the net result of these two effects is a marked increase in the output of uric acid.

In a number of experiments mostly earlier than those included in the above list the effect of muscular activity was left somewhat ambiguous, and for some time no satisfactory account of the result of such a change in the conditions could be given. It was not till a certain number of irregular results had been obtained that it appeared that the other intercurrent factor to which the irregularities were due might be the external temperature. The results of this nature, which are included in the protocols at the end of this paper, were as follows:—

9. Nov. 20, 1906. (J. B. L., v. Table IV.) Bicycling for 2 hours about 20 miles in a very cold wind gave rise to a markedly high output of uric acid at the time—39 mg. per hour—as compared with the normal average of 33, and for the succeeding 12 hours, though the exertions had only been moderate, the output was uniformly above the normal.

10. Nov. 24, 1906. (J. B. L., v. Table IV.) Bicycling from 3 to 7 p.m., about 35 miles. On this occasion there was no wind, but the air was very damp and the evaporation of sweat and, consequently, heat loss was impeded, although the temperature of the air was not high. In this case the rate of excretion was below the average. The urine for this period included that excreted in the hours from 1 to 3 o'clock, when the rate was probably the same as in the preceding period—about 30 mg. per hour—so that the rate during the exercise was probably more below the average rate than appears to be the case—perhaps as much below the average as four days before during exercise it had been above it.

11. Jan. 27, 1907. (J. B. L., v. Table V.) Bicycling for 2 hours and skating 1 hour. Exertions moderate. The air temperature was just about 32° F., but the exercise, during the hour's skating at any rate, was accompanied with heat production out of proportion to the work done. The uric acid output was below the average rate.

12. Jan. 28, 1907. (J. B. L., v. Table V.) Moderate exercise and sweating indoors, giving an output of 30 % below the average for the time of day.

13. May 11, 1907. (J. B. L., v. Table VII.) Bicycling 30 miles in the course of 4 hours, warmly dressed on a warm day. The effect of this on the output of uric acid was obscured by intercurrent factors, so that the excretion appears to be at about the normal average rate.

Such results as these suggested that it was necessary to determine, if possible, more precisely the effect, if any, of variations in the external temperature and the rate of heat loss. That the external temperature had some influence on the output of uric acid was suggested also by the figures obtained on Nov. 20 to 23 (v. Table IV). The 21st and 22nd were exceptionally warm, mild days, and the output on these two days was lower than it had been at any time since the observations began on Nov. 6. On the 23rd the first definite attempt to test this influence of heat loss was made by dressing more lightly than the subject was accustomed to do even in the height of summer. The weather at the same time turned colder. In the first eight hours, from 9 a.m. to 5 p.m., 40 mg. more uric acid was produced than corresponded to the average rate, and 76 mg. more than in the same periods on the preceding warm day.

The most striking of the experiments especially planned to test this influence of external temperature on the output of uric acid were described in an earlier paper (Cathcart and Leathes). Without going over the ground covered by that paper the experiments showed that loss of heat might increase the output of uric acid more than 70 %. If, at the same time, muscular exertions occurred the effect was much less, while if severe muscular work were done in heavy clothing the output of uric acid during the period of the exertions almost disappeared, the figure 3·6 at 7 p.m. on May 30 being almost entirely accounted for by the correction, 2 drops of $\frac{N}{20}$ $KMnO_4$ only being used for the oxidation of the ammonium urate obtained from 80 c.c. of urine. The full protocol of this series of experiments (May, 1907) is included among those at the end of this paper (Table VIII).

A less-marked result, but still pointing in the same direction, was obtained on another subject on Sept. 6, 1907. The result is most obvious if the rates for this day are compared, not with the average for the whole month, but with those of the preceding day.

The counterpart of these results is contained in the results of staying in a room at the temperature of 37° C.

1. Jan. 29, 1907. (J. B. L., v. Table V.) The subject stayed in a hot room at 37° C. for 5 hours with, after an hour, an interval of half an hour for

the meal due at 1 o'clock. The output at both 1 o'clock and at 5 was 27 mg. per hour, the average rates being 32 and 31 mg. respectively.

2. Sept. 26, 1907. (J. B. L., v. Table XI.) The same experiment, but for 3 hours only. The output during this period, including these hours, was at the rate of 29 mg. per hour, whereas in the preceding and in the following period the rate was 36 mg. per hour.

The effect in both these experiments was not very pronounced, much less than during muscular work which is sufficiently active to raise the body temperature. But even in the first of these two experiments, when the stay in the hot room was longer than on the second occasion, the body temperature rose only 1° F.

It seems, therefore, possible to account for the different effects on the uric acid output that may be observed during the carrying out of muscular work as dependent upon the influence of heat loss or heat production. Rapid movement through cold air tends to increase the output, whereas the heat produced in muscular work tends to lower it, and the actual rate to be observed in any particular instance depends upon which of these two influences predominates.[1]

The later effect of muscular work, coming on after the work is over and lasting it may be for 48 hours or more, is the more remarkable of the two. It might be regarded as due to protracted excretion of the uric acid which was produced during the time when the muscles were active. But this is not the view that we take. We have failed to find any evidence for the idea that uric acid is apt to be retained in a normal individual.

Some of the arguments which led us to reject the idea that the normally low output at night is due to retention are capable of general application. But the most striking evidence on this point is furnished by the curve of uric acid excretion during a febrile rise of temperature which was published in an earlier paper (Leathes). That curve shows that as soon as the temperature begins to rise about 5 hours after a dose of typhoid vaccine the excretion of uric acid rises too, reaches its maximum when the temperature is at its maximum, and declines as the temperature declines. The maximum rate of excretion occurs at exactly the time when normally this rate is at its lowest, when, according to Hirschstein, uric acid is apt to be retained; and the decline occurs at the time when normally there is a rise, and, according to Hirschstein, the retained uric acid is flooded out. In this case, at any rate, it is clear that the rate of excretion of uric acid varies very closely with the rate of formation; it accompanies and runs parallel with the curve of body temperature, no less closely than the temperature curve corresponds to the tide of subjective sensations. There is not the slightest sign of retention or delayed excretion.

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[1] On May 9, 1907 (v. Table VII) another and different effect of cold from the one described was obtained. On this occasion the measures adopted for effecting loss of body heat were far more energetic than in any of the other experiments we have done. The subject sat in a cold bath without moving and was continually sponged with fresh cold water for 70 minutes. The rectal temperature, which was 100·3 at the beginning of the treatment, had fallen 4° F. by the end of it. The output of uric acid was reduced 20% for 48 hours after this. The experiment has not been repeated because it was evident that the range of rates of heat loss within which physiological adaptation is possible had been considerably overstepped. Excessive stimulation seemed to result in depression.

The amount of uric acid excreted over and above the normal average output in 48 hours, after severe muscular exertions of September 10, in the third of the experiments referred to above was 0·27 g., a very marked and considerable increase. But after a meal containing 173 g. of thymus in the experiment described in the first part of this paper, nearly twice as much, or nearly half a gramme of additional uric acid, was excreted in 28 hours, and at the end of that time the output was at the normal rate again. If, therefore, the 0·27 g. which resulted from muscular activity had been all produced during the work, the results of the thymus feeding experiments would lead one to expect it to be all excreted, at any rate, within 16 hours, or a third of the time over which its excretion was actually spread out.

It seems, therefore, probable that muscular activity intensifies the activity of certain processes which lead to the formation and excretion of uric acid, and causes this intensified activity to be maintained for a considerable time after the muscles have stopped working.

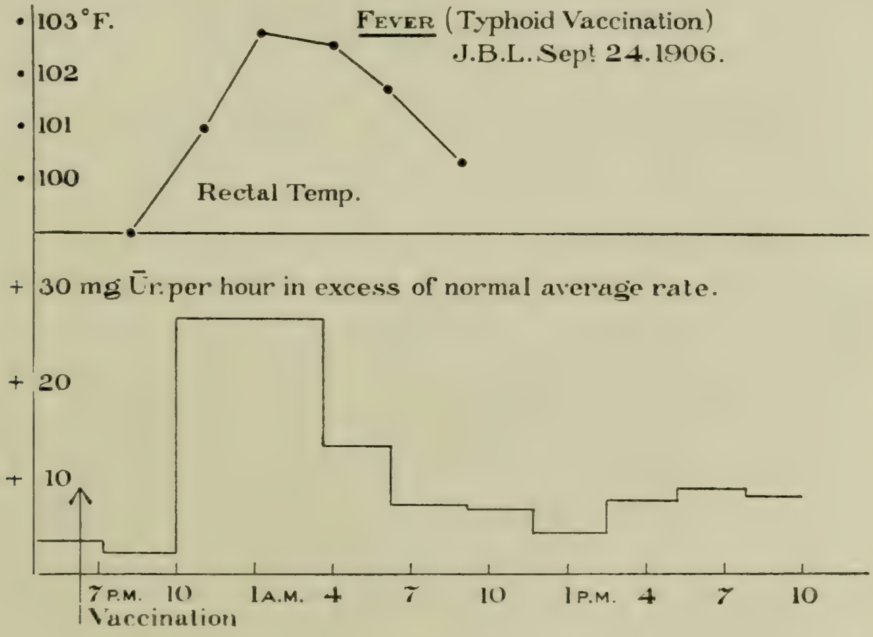

FIG. 2.

In the Figs. 2, 3, 4, and 5 the curves show the course of the acceleration in the rate of excretion of uric acid over and above the normal average rates. The zero line in these curves represents the normal average rate during each of the several periods of the day indicated by the numbers below this line. The ordinates of the curve give the excess of uric acid excreted during and after fever, exposure to cold, or muscular work as indicated in each figure. These curves serve to illustrate the points under discussion. They show that in fever (Fig. 2) the maximum rate is attained when the temperature is rising and at its height, that as the temperature returns to normal the output of additional uric acid is diminished, though the normal rate is not re-established till more than 24 hours have passed: that muscular work (Figs. 4 and 5) causes an increased output which begins only after the work has stopped, at any rate if the work does not last longer than in these experiments, but is then maintained at a level considerably above the normal for many hours.[2] Lastly, Fig. 3 shows how the

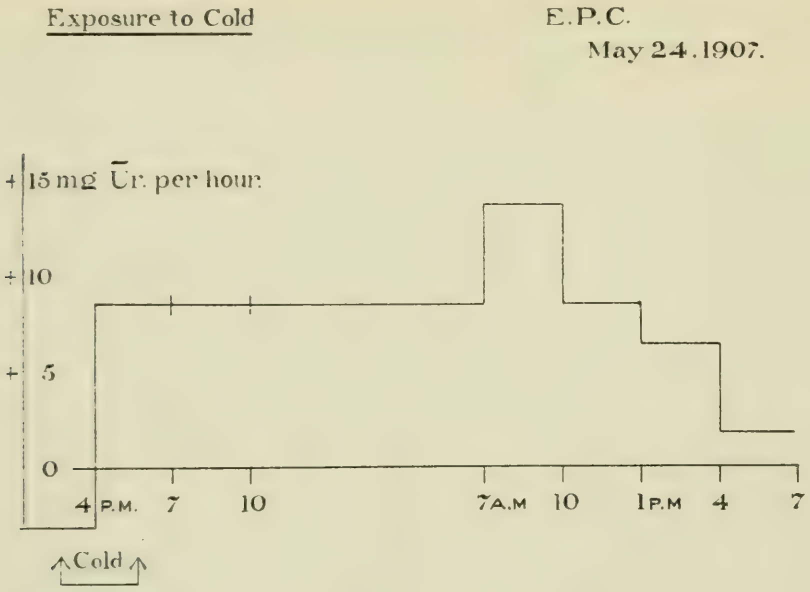

FIG. 3.

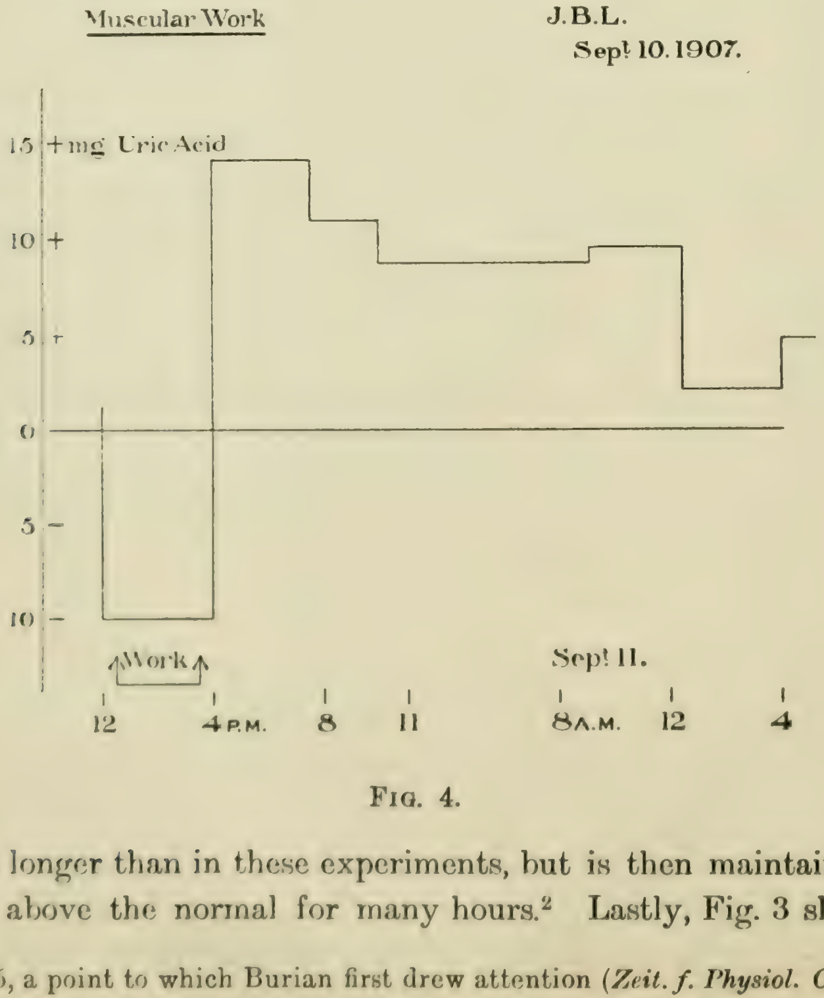

FIG. 4.

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[2] In Fig. 5, a point to which Burian first drew attention (*Zeit. f. Physiol. Chem.*, xliii. 533) is confirmed and illustrated. He states that muscular activity is followed by an increased

reaction to cold, when no voluntary muscular activity occurs, results in an increased output of uric acid that comes into full evidence during the exposure, and then is maintained for many hours after the abnormal loss of heat has been stopped.

What then is the nature of the processes the activity of which, as manifested by an increased uric acid output, is intensified by muscular work? The fact that the output during the work tends to be diminished, and especially the fact that the subsequent increase lasts for a time far longer than, as we have seen there is ground for supposing, could be accounted for by delay in excretion, seems to exclude the probability of the activity concerned being the voluntary contractions of the muscles involved in the performance of the work. And yet when a change in the condition of the neuro-muscular system results in a change in the amount of excretory products of metabolism it is necessary to assume that these products are derived from the muscles till it is proved that that is not the case. We cannot leave the muscles out of account till, for instance, it is shown that the metabolism of muscles that are not at the time actually doing work may not be intensified by an antecedent period of activity in the form of work, the metabolism, for instance, that finds its expression in the constant condition of tonus; or, again, till we can say that the work has not led to an increased rate of decay in the fabric of the tissue that has been primarily affected by the work.

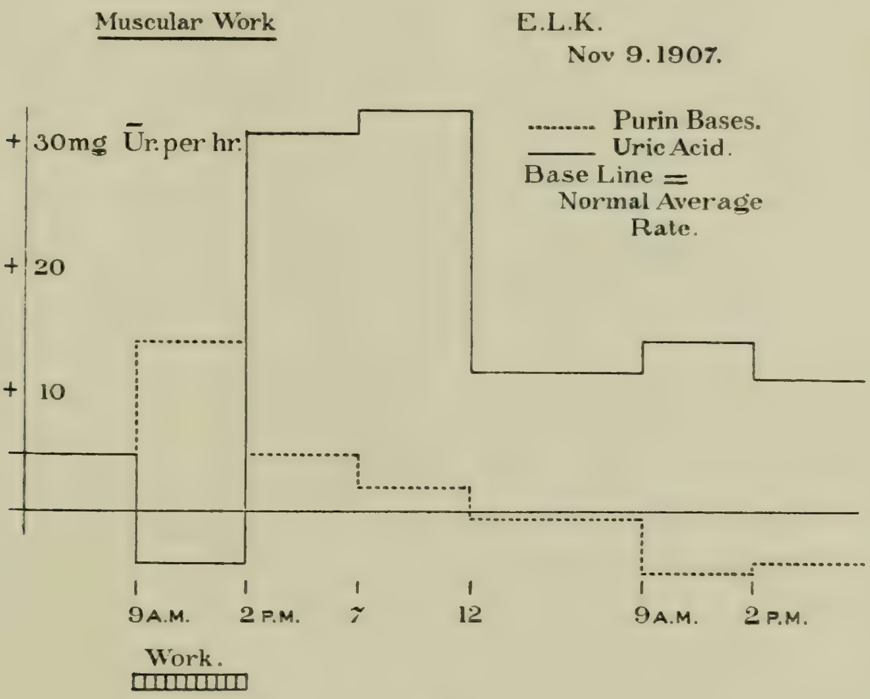

FIG. 5.

output of purin nitrogen, at first in the form of purin bases, subsequently in that of uric acid. The dotted line in Fig. 5 shows the course of the excretion of purin bases other than uric acid, the curve being referred to the normal average output represented at each period of the 24 hours as the horizontal base line. It is raised during the work, but sinks as the uric acid curve rises. The same point is illustrated again on Dec. 1 in the same series of experiments (Table X).

Till these possibilities are excluded there can be no warrant for ascribing the increased output of uric acid to any other tissue than the muscles.

The condition of muscles which are not actually doing work is not one of suspended animation; they are the seat of metabolic change, and, at any rate, one physical manifestation of this metabolism is the state of tonus which is characteristic of normal voluntary muscles, and is subject to variations that are regulated through the nervous system.

Then, again, we have seen that the effect of muscular activity on the output of uric acid is modified by the rate of heat loss and heat production. Muscular work is not commonly accompanied by such an acceleration of the rate of heat loss as to leave the body temperature unaffected; the net effect is to raise the body temperature: when this is the case the output of uric acid tends to be temporarily lowered. But when the rate of heat loss is abnormally great, then, in spite of the heat produced in the working of the muscles, the rate of uric acid production during muscular work may be increased. Again, when the rate at which heat is given off is increased, and compensatory heat production by voluntary exertions is not allowed to occur, then there may be a great increase in the output of uric acid, that occurs at once during the exposure to cold, and suggests that the reaction of the body to cold is a form of activity chemically indistinguishable from that which follows muscular work. In this reaction of the body to cold there are signs that the neuro-muscular apparatus is the seat of intensified activities other than voluntary contraction; shivering occurs, and this, as also the subjective feelings of briskness and the condition referred to as the bracing effect of cold, must have this significance. In fever, too, the rise of temperature is ushered in by signs of the same kind; the shivering fits are unmistakable evidence of an exaggerated activity in processes within the muscles quite different from those that manifest themselves in the voluntary contractions by which muscular work is carried out; and here, too, the output of uric acid is instantly increased. Other evidence of increased activity in processes within the muscles, different from those which show themselves in voluntary contraction, is found in the fact pointed out by Hale White that the axillary temperature on the paralysed side of a hemiplegic patient is often higher than on the sound side, although, as he has shown, in such a case the loss of heat may be greater on the paralysed side.

These considerations suggested that the normal diurnal variation in the output of uric acid, the minimum at night and rapid rise to a maximum in the morning hours, might be due to a tide in the activity of neuro-muscular functions, of which the tendon reflexes are a measure. During sleep reflex activity is at a low ebb; the rest of sleep gives rise to refreshed energy in such activity, and as the day advances the refreshing effect of sleep wears off. Curves of the variations in the intensity of the tendon reflexes were constructed by Lombard, which show precisely the character and form that such considerations would lead one to suspect, and the curves of diurnal variations in the output of uric acid have the form which would be required, if the principal variant cause in the production of endogenous uric acid were such functions as those typified in the neuromuscular phenomenon of the tendon reflexes.

We have endeavoured to test the influence of muscular activities of this kind by observations on the effect of doses of strychnin upon the output of uric acid. The experiments were as follows:—

1. Nov. 8, 1906. (v. Table IV.) 0·5 c.c. Liq. Strychninae Hydrochlor. was taken at 1 p.m., and again at 5 and 9 p.m., and at 9 a.m. the next morning. In the 24 hours immediately preceding the first dose the output had been 0·604 g., in the 24 hours following the first dose it was 0·629 g., and in the next 24 hours 0·596 g. No appreciable effect, with the exception of a slightly increased output during the night amounting to about 15 mg., was detected as a result of taking 20 mg. of the salt in four doses spread out over 20 hours.

2. Nov. 18, 1906. (v. Table IV.) 1 c.c. Liq. Strychninae Hydrochlor. was taken at 1 p m., and again at 5 p.m.—20 mg. in two doses, with an interval of 4 hours. In the 24 hours preceding the first dose 3 doses of sodium citrate had been taken, each of 5 g., causing a high output amounting to 0·644 g.; in the 24 hours following the first dose of strychnin this was further increased to 0·671, and in the succeeding 24 hours the output was 0·675 g., of which, however, 25 mg. was, probably, due to the two hours' ride in a cold wind at the end of the period. The average 24 hours' output during the three weeks throughout which the diet was taken was 0·626 g., and these figures, for the 48 hours after the first dose of strychnin, were the highest observed at any time in the course of the experiment. The result is, however, open to the objection that the action of the citrate may have been in part responsible for some of the high rates in the first 24 hours.

3. Feb. 19, 1907. (v. Table VI.) The same subject took 1 c.c. of the same solution at 5 p.m., and again at 10 p.m., and on the next day also at 9 a.m. and 1 p.m. In the course of this day there were some subjectively perceptible signs of the action of the poison, 40 mg. having been taken in four doses spread over 20 hours. In the 24 hours preceding the first dose the uric acid excreted amounted to 0·507 g.; in the 24 hours during which the strychnin was taken, beginning with the first dose it amounted to 0·481 g., and in the next 24 hours to 0·573 g.—again the highest figure for any period of 24 hours in the course of this experiment.

4. Nov. 17, 1907. (v. Table X.) Another subject took at 12 and 5 p.m. 10 mg. of strychnin nitrate, and again the same dose at 9 a.m., 12 and 4 p.m., in all 50 mg. in five doses, spread over 28 hours. Symptoms of increased reflex excitability were noticed in the evening of the 18th. In the 24 hours preceding the first dose the uric acid excreted amounted to 0·389 g.; in the 24 hours, beginning 2 hours after the first dose, it rose to 0·490 g., but in the succeeding 24 hours, at the beginning of which the last dose was taken, it fell to 0·423 g. The average normal output in 24 hours during this experiment lasting more than 4 weeks was 0·429.

5. Nov. 23, 1907. (v. Table X.) This subject took again 3 similar doses

of the same solution of the nitrate of strychnin at 12, 3.30, and 8.30 p.m., and 2 more on the next day at 12 and 3.30 p.m. In the 24 hours preceding the first of these doses the output of uric acid had been abnormally high—0·476 g.—as the result of taking a large dose of sodium bicarbonate on the evening of the 21st. During the 24 hours after the first dose it was correspondingly low—0·349 g., but during the 24 hours beginning an hour and a half before the last dose it rose to 0·483.

In all these experiments, except the first where the dose was smaller and spread out over a longer period than in the others, a considerable dose of strychnin was followed by a marked rise in the output of uric acid.

Taking, therefore, all this evidence together, it seems clear that the voluntary muscles are the seat of activities other than voluntary contractions which set up metabolic changes that are characterized by an increased output of uric acid, and that some of the most striking variations in the rate at which endogenous uric acid is produced in the body are accounted for by these processes and traceable to the muscular system. The processes come markedly into evidence in the reaction to cold, and also in the rise of temperature in fever, and the facts suggest that thermogenetic processes in the muscles are prominent among the functions in the execution of which uric acid is produced.

If such a view of the origin of a considerable portion of the endogenous uric acid be adopted, it naturally occurs that evidence in support of it or to the contrary should be forthcoming from the study of the output of uric acid in various pathological conditions in which the neuro-muscular functions are abnormal. But a difficulty arises from the fact that the conditions that come in question are, with few exceptions, chronic, and not recovered from, so that it is not possible to obtain from the same individuals figures under normal conditions. All that it is possible to do is to determine the daily output per kg. of body weight, and compare this with the values obtained from normal individuals. But these latter, as the protocols at the end of this paper show, are very variable. In the following list is given the number of mg. of uric acid excreted daily per kg. of body weight on purin free diets on different occasions by normal subjects in our experiments:—

J. B. L.	Sept. 1906.	Insufficient diet . . .	6·2
,,	Nov. 1906.	Abundant diet . . .	8·9
,,	Feb. 1907.	,, ,, . . .	7·1
,,	May 1907.	,, ,, . . .	7·5
,,	Sept. 1907.	,, ,, . . .	9·3
E. P. C.	May 1907.	,, ,, . . .	4·0
E. L. K.	Nov. 1907.	,, ,, . . .	5·9
H. S. R.	Jan. 1907.	,, ,, . . .	7·4

From these figures it appears that there are very wide individual variations in healthy subjects, and that anything between 4 and 9 mg. per kg. may be normal, and that even in the same individual on different occasions there may be variations amounting to nearly 50 %. These facts make it difficult to argue from the figures obtained from a patient except when it is possible to make observations on him under normal conditions as well as abnormal, keeping the diet the same throughout.

Nevertheless, the figures furnished by Spriggs are of very great interest. Among the cases studied by him are two of tetanus : one of them was a man of about 82 kg. in weight who, while having spasms, excreted 10 mg. of uric acid per kg. per diem on a purin free diet, and later, when the spasms had passed off and the diet included fish and meat, excreted only 8 mg. per kg. This patient then excreted 25 % more uric acid during the spasms, in spite of the fact that as he recovered he took more food, and food containing precursors of uric acid. The other case, a boy of 11, weighing 25 kg., excreted 20 mg. per kg. during his attack, which, as Spriggs points out, is probably excessive even at his age. No data are given as to the output normal for this subject on a similar diet after recovery.

Two cases of spastic paraplegia were also examined by Spriggs : one of them excreted 6 to 7 mg. per kg., the other 7 to 9. In both cases there seems to have been some suspicion of incontinence, which may cause the figures to be lower than they should, but in any case it is impossible to say that in regard to this condition they turn the scale either one way or the other, owing to the impossibility of getting controls.

On the other hand, Spriggs gives figures from cases in which, according to the results of our investigations, low values might perhaps be expected, but in which normal or even high values were observed. Thus, in a case of tabes with marked hypotonicity of the muscles, on three successive days the output was 9, 7, and 9 mg. of uric acid per kg., and in a boy of 4, with amyotonia congenita, it averaged 17 mg. per kg., when in another boy of the same age, who was normal, it was found to be 14 mg. per kg. Also in two cases of muscular dystrophy with remarkably low creatinin output the output of uric acid was high, ranging from 11 to 13 mg. in a boy of 16, and from 9 to 12 mg. in a man of 22 ; and in a case of myasthenia gravis also with a low yield of creatinin, the uric acid averaged 8 mg. per kg. daily. These last cases with loss of muscular power are perhaps less significant than the hypotonic cases, if we are right in associating the increased output of uric acid that we have found to follow muscular work, not so much with the work itself, as with the heightened muscular metabolism of other kinds that the work sets up for some time afterwards. And the significance of the cases of pathological hypotonicity may be regarded as emphasizing the difficulty that there is in defining exactly the relation between chemical change and functional activity. The fact that in health a particular form of chemical change is found to be associated with a particular form of functional activity does not imply, as a matter of course, that impairment of that function in disease must necessarily be associated with diminished chemical change. An efficiently working machine requires oiling and fuelling in proportion to the rate at which it is worked, but a machine that is out of order does not necessarily take less oil and consume less fuel in proportion to its inefficiency. It is just in the disturbance of the normal ratio between effect on the one hand and friction and fuel consumption on the other that its inefficiency is made manifest. We are, therefore, we think, justified in attaching more weight to the positive evidence in support of our views supplied by Spriggs's cases of tetanus than to the negative evidence furnished by the other cases.

Spriggs himself is inclined, however, rather to associate the endogenous uric acid with visceral functions, without prejudice as to what the viscera concerned may be. Hirschstein more definitely ascribes as much as 70 % of it to the activities of the digestive organs. This view, as pointed out before, is not in harmony with the view for which he contends in another part of his paper, but is based on the following evidence. He found that proteid food, as is well known, although free from purin compounds, causes more uric acid to be excreted the more abundant it is; that a patient in bed during one day and without food excreted considerably less than when taking food whether up or in bed; that purin bases may be found in the intestine of a dog, in undetermined but 'not insignificant amounts', three or four hours after an isolated purin free meal; and he cites the observations of Schittenhelm, who found purin bases to be present in human faeces when the diet was free from purin compounds, also in meconium, in the contents of a pancreatic cyst, and in the substance of the intestinal wall.

Our attention was diverted from the digestive organs as a probable seat of activities involving formation of uric acid in any considerable quantity for the following reasons:—

(1) The observation that complete abstention from food on one day, when the same amount of fluid was, however, taken, and at the same times as on the previous days, did not affect the form of the curve of uric acid excretion on that day. More particularly on that occasion, Sept. 22, 1906 (v. Table III), the morning rise was at least as well marked as usual. During the period ending at 4 a.m., the rate had been 8·1 mg. per hour; although the 7 a.m. meal was not taken, it rose in the period from 7 to 10 to 19·3 mg. per hour. The average rates for these periods on the four previous days had been 10·9 and 21·2 mg. per hour respectively. The lowest rate at any period on the 22nd was 17 mg. per hour. It is true that in the 24 hours from 7 a.m. on the 22nd to 7 a.m. on the 23rd the output was only 349 mg., when the average was 435, and that in the succeeding 24 hours it rose to 422 mg. But this fact seems to be less significant than the fact that the morning rise occurred as sharply, and the general form of the curve for the day was the same as when food was taken.

This seemed to show that the ordinary diurnal tide of uric acid excretion was independent of the activities of the digestive organs, though the forms of activity on which it does depend may, when no food is taken, tend to be depressed. Hirschstein also notes that his patient, even on the day which was spent in bed and without food, excreted more uric acid during the morning than during the previous night.

(2) Hopkins and Hope found that isolated meals of non-nitrogenous food gave rise to no increased excretion of uric acid, although under these circumstances a definite leucocytosis could be observed, and although isolated meals of purin free nitrogenous food caused a definite increase in the rate of uric acid output.

(3) In the most marked changes in the rate of uric acid excretion that we were able to bring about experimentally, there was no reason to think of the digestive organs as being particularly concerned. In the experiments on the effect of fever, a strong disinclination for food was experienced, and it was only with difficulty that the ordinary rations could be taken. After muscular work no more food was taken than at other times, and there was no reason of which we were aware for thinking that the food was imperfectly dealt with before, or more perfectly after the work.

But although our attention was by these considerations, and the results of our experiments, turned in other directions, we do not pretend that the kinds of activity which we have found to be associated with the formation of endogenous uric acid are solely responsible for it.

Nevertheless, the few experiments which we have carried out expressly with a view to determining the relation of digestive activity to endogenous uric acid, have given results which are not compatible with the view that digestive activity is very closely related to its production. On a number of occasions the subjects of the experiments omitted to take the first meal of the day, taking instead the same amount of water as was usually taken in the form of milk. On four such occasions the result was that the usual morning rise in the rate of uric acid excretion did not appear, which at first sight suggests that this morning rise is the result of the activity of the digestive organs brought about by taking breakfast. But if that were so, the morning rise should be merely postponed, and should appear in due course after the first meal taken later in the day; but this was not the case. The output remained low throughout the day, although it was only the first meal that was dropped. The dates and figures were as follows:—

Sept. 21, 1907	8.30 a.m. to 12.30 18 mg. per hour instead of average 32	12.30–4.30 p.m. 14 mg. instead of average 30	
Nov. 16, 1907	9 a.m. to 2 p.m. 10 mg. per hour Average 23	2 p.m. to 7 p.m. 15 mg. per hour Average 21	7 p.m. to 12 13 mg. per hour Average 17
Nov. 20, 1907	11 mg. per hour Average 23	11·5 mg. per hour Average 21	7 mg. per hour Average 17
Nov. 29, 1907	12 mg. per hour Average 23	14 mg. per hour Average 21	12 mg. per hour Average 17

On the first and last of these days, Sept. 21 and Nov. 29, the subject stayed in bed till the middle of the day when the first meal was taken, on the other two days he was occupied in the laboratory as usual. Other experiments (June 2, Sept. 29, and Nov. 30) showed that staying in bed, if breakfast was taken at the usual time, did not interfere with the occurrence of the usual morning rise of uric acid output.

On other occasions on which the first meal was omitted a different result was obtained: the rate of output rose in the usual way in the morning in spite of the fast:—

Sept. 27, 1907	8.30 to 12.30	12.30 to 4.30	4.30 to 8.30
	34 mg. per hour	26 mg. per hour	33 mg. per hour
	Average 32	Average 30	Average 31
Nov. 19, 1907	2 p.m.	7 p.m.	12 midnight
	24 mg. per hour	17 mg. per hour	17 mg. per hour
	Average 23	Average 21	Average 17

This result is, of course, equally, though in a different way, difficult to reconcile with the view that the morning rise signifies merely the return of the organs of digestion to activity. Why the results were in these two cases different from those referred to above we cannot with any confidence suggest. But on the two days before Nov. 19 considerable doses of strychnin had been taken, and the effect that we have observed this drug to have upon the output of uric acid may last for 24 hours. It is possible, therefore, that the result of Nov. 19 was the result of the action of the strychnin.

On Sept. 28, when active muscular exercise in the open air was taken before the first meal at 12.30, and on Dec. 1, when severe dumb-bell exercises for two hours were performed and no food taken till three hours after the work, the effects were those which muscular activity gave on other occasions when food had been taken.

It would seem, therefore, from these results, that the ordinary diurnal tide in the excretion of uric acid is determined by some form of activity other than that of the digestive organs, but some form of activity which is liable to be depressed if food is not taken at the habitual time.

Moreover, if we are right in associating the daily fluctuation in the rate of output of uric acid with a daily fluctuation in the activity of muscular functions, such as is indicated in the curve of variation in the intensity of the knee jerk obtained by Lombard and referred to above (p. 428), it may be noted that this latter curve, in addition to the main crest appearing in the early hours of the morning, shows minor ones shortly after each meal. It is possible for a function to be intensified by taking food and give rise to metabolic products in increased amount after food, without making it necessary to refer these products to the digestive organs as the seat of their formation.

The only other experiment at all bearing on this point was an indecisive one. On Nov. 27, 1907, 30 mg. of pilocarpin nitrate was taken in the evening. Sweating and salivation followed, but if there was any increased activity in other glandular organs it did not manifest itself in any marked departure from the normal figures for uric acid excretion. Possibly if the urine had been examined at shorter intervals some more definite effect might have come to light.

The possibility of the involuntary muscular functions of the alimentary canal being in some way related to the formation of endogenous uric acid was tested on Sept. 23, 1907, by taking 0·5 g. of calomel at night. The purgation that followed, which was fairly sharp, was marked by no pronounced change in the output of uric acid, though there was a tendency for the figures to be low for more than 24 hours.

Some indication of the activity of other visceral functions than those of the secreting glands concerned in the digestion of food may be obtained from the excretion of nitrogen and phosphorus. The intake of nitrogen and phosphorus, though uniform throughout the day in all our experiments, was stopped for several hours at night. The food metabolism that results in the excretion of urea is, therefore, lowest in the morning, and rises with each meal to a maximum late in the day, or as shown in the experiment of November, 1906, and commented on in an earlier paper (Leathes), in the early part of the night during sleep. This curve is so entirely different from that for the excretion of uric acid, the maximum and minimum of the one coinciding approximately with the minimum and maximum of the other, that we feel great difficulty in associating the endogenous uric acid with the internal metabolic processes of viscera concerned in assimilation. The curve for phosphoric acid is, broadly speaking, similar to that for total nitrogen, the only noticeable point being that there is a tendency (November, 1907) for greater delay in the removal of superfluous ingested phosphorus than of superfluous nitrogen, so that the minimum rate of phosphorus excretion may occur after the rate of nitrogen excretion has begun to go up as a result of the morning meals.

The amount of phosphorus excreted in our experiments was large owing to the large amount of casein taken, and in so far as the fluctuations in the rate at which it was excreted indicate fluctuations in the intensity of visceral processes concerned with food metabolism, they do not point to uric acid being produced by these same processes.

When, however, we infer from the results which we have obtained, that there are metabolic changes in the muscles that are largely concerned with the production of uric acid, and that the daily fluctuations in the rate at which it is turned out bear no relation to the activity of digestive processes, we do not wish to imply that all the endogenous uric acid is derived from the muscles. But if the muscles are concerned and other organs as well, when the relative bulk of the muscles and the other organs of the body is considered, and also the small absolute amount of uric acid produced as a result of even the most intense activity of the muscles, it is not to be wondered at that only those variations can be detected which may be traced back to the voluntary muscles, forming as they do 40 % of the whole body.

There is another way in which the experiments we are recording show variations to occur in the output of uric acid. Some of the series of figures given were obtained from the same individual, on the same diet, on different occasions: as the following list shows, the output varied considerably:

In Aug. 1906 the average output in 24 hours was 610 mg.
„ Nov. 1906 „ „ „ 626 „
„ Jan. 1907 „ „ „ 560 „
„ Feb. 1907 „ „ „ 500 „
„ May 1907 „ „ „ 527 „
„ Sept. 1907 „ „ „ 652 „

The low figures obtained in the early months of 1907 attracted attention at the time, and were hypothetically referred to the condition of the subject, who had been in town from August of the preceding year until the end of March, and attributed the fact that he was at the time more subject to catarrhs and sore throats than usual to this circumstance. It was a matter of interest, on this supposition, to see what the output would be in September after a holiday of six weeks in the mountains. The result that the average output was higher in this month than on any previous occasion tends to support the view that, other things being equal, the amount of uric acid excreted will be highest when the general health is best.

It is true that in February and May the diet contained 1 pint less milk than in the other four series, which means that about 10 % less proteid was taken daily, although the nitrogen intake and calorie supply were still abundant. Now diminution of the proteid in the food is accompanied, as is well known, by diminution in the output of uric acid. But in the experiment of Sept. 1906, when the nitrogen intake in the same subject was reduced not merely 10 % but almost 75 %, and the average daily nitrogen deficit from the urine analysis alone amounted to more than 2 g., the output of uric acid fell only to 450 mg.: this makes it extremely improbable that when it fell to 500 in February, the fall should be entirely ascribed to the fact that the proteid supply was 10 % below what it was when the output was 650 mg. There was some other factor to account for the low output in February and May, and that suggested in the previous paragraph is the one to which we are inclined.

Before concluding, there are some other particulars in our experiments to which reference may be made. The effect of a weak infusion of tea, and of a solution of 60 mg. of caffein taken at 7 p.m. on November 12 and 13, will be found in Table X. These drugs were tested because of the action which they, in common with other purin derivatives, have upon the muscles. On both occasions the output was low during the following night, but whether this should be referred to the drugs these isolated observations cannot decide. But, at any rate, on neither occasion was there any sign of an opposite effect at any time, and that was the point which we wished to test.[3]

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[3] Krüger and Schmid, *Zeit. f. Physiol. Chem.*, xxxii, 104, found that as much as 200 mg. of caffein had no effect on the 24 hours' output of uric acid.

On November 14, in the same series, 14 g. of lactic acid was taken, dissolved in the necessary amount of soda at 7 p.m., with a view to confirmation of Wiener's experiments on the animal synthesis of uric acid. In the 24 hours beginning at midnight, the output was hardly above the average, though considerably above that for the preceding and subsequent 24 hours. Wiener found 10 g. sodium lactate to give a larger increase in a man than 17 g. did in our experiment. On November 21, the corresponding amount of sodium bicarbonate gave rise to an output in the following 24 hours increased, in comparison with that for the preceding 24 hours, as much as it had been when the lactate was taken. It would be difficult, therefore, to ascribe any increased output to the lactic acid itself.

Like sodium bicarbonate, sodium citrate (cf. November 18, 1906, Table IV) seems, as was found by Macleod and Haskins, to increase the amount of uric acid. But the action of salicylate of soda is on a different scale: 4·7 g. at 7 p.m. on November 25 (Table X) gave rise to the appearance within 24 hours of 0·77 g. when the normal average amount was 0·43. This has been observed before by many others (Jackson and Blackfan, Rockwood and Epps, and references in von Noorden's *Pathologie des Stoffwechsels*).

Potassium bromide, of which 10 g. was taken in the course of November 15, 1906 (Table IV) was found to have absolutely no effect on the output of uric acid.

Lastly, the long series of observations upon the excretion of creatinin calls for comment. No determinations of creatin were made. But the one remarkable feature about the excretion of creatinin is its extraordinary constancy. The only occasion on which there was any marked departure from the normal rates was during the fever of September 24, 1906 (Table III), which has been commented on in an earlier paper (Leathes). In some cases small increases were noted after muscular work: on September 10, 1907 (Table IX), an increase over the normal average for 24 hours of 0·14 g.: on the three days following the experiment of May 30, 1907 (Table VIII), the output was 1·57, 1·56 and 1·54 g. when the normal average was 1·49 g., and on May 11, 1907 (Table VII), an additional excretion of 0·12 g. after exercise was observed: on this occasion considerable quantities of additional fluid were drunk in the form of soda-water, about 1500 c.c. These variations are trifling compared with those affecting the uric acid under such circumstances. Also, in the six different series of observations on the same individual, including the experiments on a low nitrogen intake in September, 1906 (Table III), the output was remarkably constant at about 2·0 g.

CONCLUSIONS

1. A marked increase in the output of endogenous uric acid has been found to occur in three conditions: (*a*) Fever, (*b*) exposure to cold, (*c*) after severe exertions. The increased output coincides and terminates with the febrile rise of temperature, coincides with and outlasts by many hours the exposure to cold,

follows the exertions and lasts for many hours after them. It is suggested that in all these three conditions the uric acid has its origin in metabolic processes occurring principally in the voluntary muscles, and not immediately related to voluntary contractions and work.

2. The daily tide in uric acid excretion, high output in the morning, low output at night, is not due to retention of uric acid formed during the night; nor is it due to the fact that the digestive organs are inactive during the night, if that is a fact, with the last meal taken at 9 or 10 p.m., as in most of our experiments. It is rather due to the quickened activity of all functions, especially those of the voluntary muscles, which results from the rest of sleep.

3. Generally speaking, the more lively the performance of the functions of the body as a whole, the greater the amount of uric acid produced will tend to be.

PROTOCOLS. TABLES III–X

Uric acid was estimated in the experiments given in Tables III and IV by Wörner's modification, in the rest by Folin's modification of Hopkins's method: the methods were checked against one another.

Creatinin was estimated by a modification of Folin's colorimetric method, which was checked against the method as carried out by Folin himself.

Total purin nitrogen in the experiments given in Table VII was estimated by the Camerer Arnstein method; in the experiments in Table X the estimation was carried out after filtering off the ammonium urate for uric acid estimation; the total purin nitrogen was then obtained by addition of the uric acid nitrogen.

Phosphoric acid was estimated by titration.

All the uric acid and creatinin estimations were done by the same worker (J. B. L.); the other estimations in Table VII and VIII and the first part of Table IX, all by E. P. C., and in the second half of Table IX and in Table X, all by E. L. K.

The experiments were all carried out at the Lister Institute for Preventive Medicine.

TABLE III

N. = g. Nitrogen excreted per hour. C. = g. Creatinin excreted per hour. U. = mg. Uric Acid excreted per hour.

Date.	ca. 4 A.M.			7 A.M.			10 A.M.			1 P.M.			4 P.M.			7 P.M.			10 P.M.			Total in 24 hours			ending	
	N.	C.	U.	N.	C.	U.	N.	C.	U.	N.	C.	U.	N.	C.	U.	N.	C.	U.	N.	C.	U.	g. N.	g. C.	mg. U.		
1906, Sept.																										[J. B. L.] Diet started 13th. Six meals at 7 and 10 a.m., 1, 4, 7, and 10 p.m., each of 90 g. 'Arrowroot' biscuits, 20 g. butter, 350 c.c. water. = ca. 35 Cal. per kg. and 5·7 g. N. per diem.
14																						12·4	2·1	490	11.15 a.m.	
15																						8·9	2·0	432	10 a.m.	
16																						7·7	2·2	444	10.30 a.m.	
17										20	84	24	34	93	18	44	91	24	31	80	21	7·7	2·1	454	10 a.m.	
18	34	58	8	32	86	14	24	82	20	39	87	23	40	80	20	28	90	18	23	81	14	7·7	1·9	380	10 p.m.	
19	35	84	14	17	85	16	18	78	19	40	92	26	49	94	24	41	87	19	26	83	14	8·0	2·1	440	,, ,,	
20	31	74	9·5	22	85	13	22	86	23	31	94	27	32	94	23	37	94	18	22	85	15	7·0	2·0	392	,, ,,	
21	39	87	12	19	80	18	20	84	22	28	99	24	26	92	25	36	93	26	29	84	23	6·8	2·1	494	,, ,,	
22	42	80	8	25	74	18	22	83	19	32	79	18	27	84	17	31	88	20	28	77	17	7·5	1·9	366	,, ,,	No food taken this day: water as [usual.
23	32	68	8·5	32	75	8	37	67	11	59	83	25	48	84	19	51	88	18	30	82	17	9·7	1·8	348	,, ,,	
24	50	80	15	28	81	19	26	87	23	28	93	28	22	80	23	19	91	23	27	83	20	7·4	2·0	504	,, ,,	4·6 c.c. Typhoid Vaccine at 6 p.m. Fever during night.
25	48	95	38	41	95	28	23	82	24	32	87	28	32	95	24	46	102	27	59	100	25	9·8	2·2	707	,, ,,	Meals dropped at 4 and 7 p.m. Water as usual. Slept from 7 to 8.30 p.m.
26	49	88	17	22	84	9	23	80	17	39	86	27	45	86	22											
Average.	40	79	14	26	83	16	24	31	20	37	88	25	36	88	22	37	92	21	27	84	18	7·95	2·0	454		
Omitting 25th & 26th	38	76	11	25	81	15	24	81	20	37	89	24	35	88	21	38	90	21	27	82	18					
																			From 18th to 21st			7·44	2·0	434		

TABLE IV

N., C., U., as in Table III.

Date. 1906, Nov.	ca. 4 A.M. N.	C.	U.	9 A.M. N.	C.	U.	1 P.M. N.	C.	U.	5 P.M. N.	C.	U.	9 P.M. N.	C.	U.	12 midnight N.	C.	U.	Total excretion in 24 hours g. N.	g. C.	mg. U.	ending at	
5				47	79	21	69	95	35	80	102	37	78	93	29	89	86	25				12 p.m.	[J. B. L.] Diet started Nov. 2. Four meals, at 9, 1, 5, and 9, each of 570 c.c. milk, 170 g. bread and 30 g. butter. = 56 Cal. per kg. and 22 g. N. in 24 hours.
6	82	71	18	33	58	22	66	87	32	81	100	35	83	94	27	74	82	26	17·7	1·98	629	,,	
7	89	79	19	53	73	22	85	81	34	92	92	31	72	83	24	68	80	22	18·2	1·96	625	,,	
8	78	83	19	46	78	21	62	97	35	75	86	28	61	82	22	55	76	21	15·6	2·03	585	,,	0·5 c.c. Liq. Strych. Hydrochlor. at 1, 5 and 9 p.m.
9	*84	*81	*23				79	83	32	100	89	30	77	82	25	68	76	19	19·6	2·08	633	,,	Do. do. at 9 a.m. *This sample collected at 7 a.m.; none at 9 a.m.
10	63	77	18	45	74	21	83	91	37	86	90	32				*79	86	25	18·1	2·01	618	,,	5 g. Sod. Citrate at midnight. *Sample from 5 p.m. till 12.
				7.30 A.M.			2 P.M.			6 P.M.			12 midnight										
11				74	72	23	62	81	32	100	93	34	75	84	22				18·2	1·94	637	,,	Urine slightly alkaline at 2 p.m.
12				76	73	18	62	—	29	95	—	30	65	—	25				17·5		622	,,	
13				71	—	21	61	—	32	70	85	32	69	77	24				16·3	—	641	,,	
14				74	77	20	68	77	36	96	84	33	76	88	25				18·4	1·94	664	,,	
15				78	80	18	66	84	35	74	77	28	71	82	24				17·1	1·95	623	,,	Pot. Bromide 2 g. at 9, 5 g. at 10.30, 3 g. at 4 p.m. = in all about 2½ drams.
	ca. 4 A.M.			9 A.M.			1 P.M.			5 P.M.			9 P.M.			12 midnight							
16	100	77	21	56	76	20	77	89	35	89	90	35	85	92	25	75	82	25	19·1	2·01	626	,,	
17	109	85	21	55	87	22	58	88	32	80	92	29	68	84	27	61	83	23	17·4	2·01	616	,,	5 g. Sod. Citrate at 5, 9, and 12 p.m.
18	134	80	27	61	72	23	66	82	33	78	84	34	64	83	28	77	79	29	17·6	1·91	679	,,	Urine alkaline till 5 p.m. 1 c.c. Liq. Strych. Hydrochlor. at 1 and 5 p.m.
19				*57	74	22	62	77	33	78	82	31	83	86	30	68	83	26	16·1	1·90	658	,,	* This sample collected from midnight till 9 a.m.
20	104	78	25	64	73	22	89	91	39	98	83	34	93	83	28	82	82	27	20·8	1·95	690	,,	Bicycling 20 miles from 10.30 to 12.30. Wind very cold.
21	90	75	17	52	68	18	66	81	29	76	81	30	75	83	25	87	77	21	17·5	1·87	563	,,	Very warm, mild day.
22	90	77	22	52	68	20	58	80	30	80	85	26	78	85	28	66	78	23	17·2	1·89	589	,,	do. do.
23	79	74	21	49	72	21	85	88	40	117	91	35	74	88	26	76	76	22	19·3	1·96	653	,,	Colder: dressed lightly in order to feel cold.
24	107	82	17	45	73	17	72	96	30				*81	92	25	55	75	23	17·2	2·00	544	,,	Bicycled gently 35 miles from 3 to 7 p.m.; not cold, but air very damp. * This sample collected from 1 to 7 p.m., and the next one from 7 p.m. to 12.
25	77	79	23	43	79	19	65	80	26	90	91	32							16·4	1·96	565	1 p.m.	
Average	95	78	21	56	73	21	71	85	33	87	89	32	76	86	27	73	80	24	17·8	1·96	626		

TABLE V

N., C., U., as in Table III.

Date. 1907, Jan.	8 A.M. N.	C.	U.	1 P.M. N.	C.	U.	5 P.M. N.	C.	U.	11 P.M. N.	C.	U.	g. N.	g. C.	mg. U.	
26				70	85	32	101	81	34	58	74	23	16·3		551	[J. B. L.] Diet started Jan. 24. Four meals, at 9, 1, 5, and 9, each of 570 c.c. milk, 170 g. bread, 30 g. butter. = ca. 56 Cal. per kg. and 22 g. N. in 24 hours.
27	58	80	13	58	86	[27]	71	85	34	60	84	19	14·5	2·00	494	Naso-pharyngeal catarrh came on on 25th.
28	71	79	14	104	92	36	89	90	[*21]	58	84	21	19·0	2·05	466	Bicycling 2 hours, skating 1 hour: 10 a.m. to 1 p.m.
29	80	81	21	65	87	27	44	87	27	70	93	22	16·0	2·08	567	Moderate exercise and sweating indoors. * Sample collected from 2 to 7 p.m.
30	59	75	20	72	92	34	85	97	36	62	75	24	15·9	1·97	638	Recovering from effects of catarrh: sat in incubation room (T. 37 C.) from 12 to 5 p.m.
31	82	79	23	78	70	32	90	79	29	[92]	[122]	[37]	18·7		644	Diet changed at 8 p.m.
Average	70	79	18	69	85	32	87	87	31	61	84	22	17·1	2·02	560	

TABLE VI

N., C., U., as in Table III.

Date.	8.30 A.M.			2 P.M.			6 P.M.			11 P.M.			Total in 24 hours			ending at	[J. B. L.] Meals at 9, 2, 6, and 10, each of 430 c.c. milk, made up to 570 c.c. with water, 170 g. bread, and 30 g. butter. = ca. 51 Cal. per kg. and 19 g. N. in 24 hours.
1907. Feb.	N.	C.	U.	N.	C.	U.	N.	C.	U.	N.	C.	U.	g. N.	g. C.	mg. U.		
14				89	133	39	100	122	28	59	88	17	18·2	2·54	511	8.30 a.m. 15th	Diet started this day
15	66	93	10	62	113	22	88	99	19	63	107	20	15·4	2·31	396	11 P.M. 15th	Catarrh.
16	56	80	14	53	89	25	70	112	25	56	87	17	13·9	2·13	457	,, 16th	
17	72	82	21	58	91	27	70	94	23	55	89	16	15·6	2·10	529	,, 17th	Sore throat.
18	71	72	19	61	101	22	59	93	22	56	91	19	15·2	2·07	500	,, 18th	
19	64	85	19	69	116	25	86	121	22	54	93	18	16·5	2·45	509	,, 19th	1 c.c. Liquor Strychninae Hydrochloratis 5 p.m. and 10 p.m.
20	41	78	15	47	104	27	72	85	26	53	109	24	11·8	2·22	515	,, 20th	Do. do. at 9 a.m. and 1.15 p.m.
21	54	73	21	70	99	30	58	96	23	57	94	17	14·1	2·10	548	,, 21st	
22	70	82	19	84	102	28	84	89	17	60	87	17	17·7	2·18	492	,, 22nd	
23	73	91	12	68	106	22	75	98	23	70	100	21	17·3	2·34	426	,, 23rd	200 c.c. water taken at 1 a.m. and 5 a.m.
24	63	95	17	59	117	28	55	94	26	64	108	21	14·6	2·47	531	,, 24th	
25	59	83	16	59	94	27	69	107	28	60	103	21	14·6	2·24	520	,, 25th	
26	71	84	17	74	107	24	56	94	17	51	93	22	15·8	2·24	464	,, 26th	0·47 Sodium Urate at 11.30 p.m.
27	53	74	17	65	98	32	58	101	24	65	106	21	14·1	2·17	[532]	,, 27th	
28	78	109	23	71	112	28	63	123	27	55	89	17	16·5	2·59	[575]	,, 28th	0·47 Sodium Urate at 9 a.m.
March 1	66	92	18	62	109	24	58	98	24								
Average	64	85	17	59	100	26	70	102	23	58	96	19	15·4	2·28	500		

TABLE VII

N., C., U., as in Table III.

B. = mg. Purin base nitrogen per hour. T.P. = mg. Total Purin nitrogen per hour P_2O_5 = mg. P_2O_5 per hour.

[J. B. L.] Meals at 8, 1, 6, and 10, each of 430 c.c. milk, made up to 570 c.c. with water, 170 g. bread and 30 g. butter

Date.	7.30 A.M.						1 P.M.						6 P.M.						10.30 P.M.						Total in 24 hours						ending at
1907. April	N.	C.	U.	B.	T.P.	P_2O_5	N.	C.	U.	B.	T.P.	P_2O_5	N.	C.	U.	B.	T.P.	P_2O_5	N.	C.	U.	B.	T.P.	P_2O_5	g. N.	g. C.	mg. U.	mg. B.	mg. T.P.	g. P_2O_5	
29																									14·3	2·10	652				9 a.m.
May 1																									14·7	1·83	415	54	192	1·94	7.30 a.m.
2							60	80	23	1·9	9·6		53	88	24	0·8	9·0	128	60	84	14	3·4	8·0	153	14·3	1·84	432	20	165	2·64	,,
3	70	80	19	2·1	8·5	139	61	90	24	2·5	10·7	57	53	81	22	3·2	10·4	125	64	84	17	1·3	7·1	156	15·0	1·98	486	48	210		,,
4	65	80	16	0·4	5·9	117	58	87	23	2·8	10·4	64	64	88	21	1·1	8·2	128	54	85	16	2·0	7·5	97	14·7	2·02	453	31	185	2·48	10.30 p.m.
5	64	81	19	3·5	9·8	168	63	81	24	1·9	9·9	76	60	76	21	2·6	9·7	126	63	97	25	1·2	9·4	154	15·1	1·99	519	60	233	3·22	,,
6	69	76	21	[9·1]	[6·0]	144	58	89	29	1·6	11·5	71	55	85	25	0·9	9·3	143	55	95	22	0·6	7·8	165	14·7	2·03	572	[98]	[289]	3·12	,,
7	67	75	20	1·1	7·7	150	66	85	31	—	9·9	84	81	89	26	2·6	11·2	156	68	84	25	3·9	12·3	178	16·7	1·97	595	36	234	3·36	,,
8	65	77	18	0·8	7·0	113	58	87	30	1·3	11·4	62	67	84	23	0·5	8·3	138	72	80	26	0·4	9·0	164	16·0	1·96	565	19	207	2·79	,,
9	86	70	23	0·3	7·9	113	80	80	28	3·4	12·9	48	81	85	[22]	2·6	10·0	[136]	75	75	[19]	2·0	8·5	[89]	18·7	1·85	593	19	217	2·69	1 p.m.
10	64	72	[13]	1·0	5·4	[97]	56	85	[18]	1·9	7·8	[38]	62	84	[21]	1·5	8·4	[74]	73	84	[10]	4·5	7·8	[103]	16·5	1·90	[419]	41	181	[2·17]	,,
11	65	84	[20]	0·7	7·5	[84]	57	88	[12]	2·9	6·7	[34]	65	97	[20]	5·1	11·8	[67]	81	88	[20]	1·8	8·4	[92]	15·4	2·01	[438]	66	210	[1·75]	,,
12	69	94	[18]	[10·4]	[6·3]	[125]	58	96	[28]	2·8	12·1	[35]													16·3	2·17	[498]	[146]	[312]	[2·11]	,,
Aver.	68	79	19·4	1·0	7·5	135	61	86	25·5	2·3	10·3	66	64	86	23·3	2·1	9·6	135	66	86	20·7	2·1	8·6	152	15·8	2·05	527	39	205	3·03	

Diet started April 30. May 9: Rectal Temp. lowered 4° F. by cold sponging from 4.15 to 5.25 p.m.

May 11 Bicycled 30 miles between 2 and 6 p.m. Warmly dressed, warm day.

Average: Bracketed figures not counted in in averages, nor figures before May 2nd.

TABLE VIII

N., C., U., P_2O_5, as in Table VII. H_2O = c.c. per hour.

[E. P. C.] Diet began May 17. Six meals daily, at 7, 10, 1, 4, 7, and 10, each of 380 c.c. milk, 60 g. bread, 20 g. cheese, 10 g. butter. = ca. 45 Cal. per kg. and 21 g. N. per diem.

Date.	7 A.M.					10 A.M.					1 P.M.					4 P.M.					7 P.M.					10 P.M.					Total for 24 hours					ending at
1907.	N.	C.	U.	P_2O_5	H_2O	N.	C.	U.	P_2O_5	H_2O	N.	C.	U.	P_2O_5	H_2O	N.	C.	U.	P_2O_5	H_2O	N.	C.	U.	P_2O_5	H_2O	N.	C.	U.	P_2O_5	H_2O	g. N.	g. C.	mg. U.	g. P_2O_5	l. H_2O	
May 18																															11·8	1·34	272	1·91	1·21	8 a.m.
19													16·1	88	36	65	57	22	101	37	88	64	18·1	169	106	89	64	18·5	168	80	16·6	1·48	371	3·05	1·55	,,
20	61	59	11·2	127	40	55	69	13·0	80	33	57	61	13·6	119	39	70	63	15·1	172	43	81	66	14·6	212	61	86	62	9·1	196	109	16·2	1·50	335	3·27	1·26	4 p.m.
21	72	60	10·6	144	71	58	61	16·4	87	48	64	65	10·6	122	46	74	63	10·9	182	50	80	65	8·6	205	61	100	71	8·8	212	113	17·4	1·49	280	3·69	1·58	,,
22	71	55	6·8	138	63	69	64	15·9	96	49	71	61	11·1	134	56	75	64	10·0	196	45	83	65	10·5	198	66	96	65	9·6	189	94	18·2	1·46	225	3·77	1·54	,,
23	63	55	6·7	133	54	72	76	19·5	108	52	64	65	13·9	148	43	74	66	12·7	206	43	78	59	12·3	203	53	93	66	14·5	185	61	17·4	1·51	259	3·74	1·38	,,
24	75	60	10·9	139	65	62	63	11·9	78	39	63	64	10·7	122	40	69	64	7·9	176	51	84	56	19·5	190	54	90	58	21·1		51	17·7	1·49	270	3·55	1·31	,,
25	77	59	17·1	153	42	66	59	28·7	164	43	72	63	20·2	145	42	83	67	17·4	200	43	85	64	12·9	202	50	94	69	17·2	196	58	18·8	1·44	475	4·0 ?	1·08	,,
26	75	59	10·8	144	59	67	65	20·4	89	46	70	61	16·1	147	49	83	67	13·7	209	51	90	67	17·1	209	76	89	67	16·7	170	79	18·7	1·51	338	3·83	1·29	,,
27	75	57	8·0	145	63	58	64	17·6	75	43	57	66	13·2	131	41	63	55	14·7	183	47	77	67	6·2	159	51	88	61	20·4	80	60	17·6	1·48	312	3·64	1·43	,,
28	74	61	12·4	132	57	67	65	20·7	96	39	67	64	19·2	173	48	78	67	19·6	215	51	81	66	12·8	218	59	95	65	12·0	227	135	17·9	1·51	369	3·33	1·29	,,
29	72	57	10·2	161	67	58	63	20·1	95	44	66	64	16·4	164	51	69	67	14·6	210	50	76	67	12·7	215	67	86	70	19·0	206	76	17·6	1·49	314	4·20	1·62	,,
30	71	56	9·8	160	78	61	61	19·0	95	54	72	68	–	146	50	61	62	8·9	178	43	52	70	3·6	132	33	95	72	6·9	66	57	17·1	1·49	311?	3·93	1·58	,,
31	75	62	9·2	176	43	65	65	13·8	108	37	71	64	11·0	170	38	90	66	11·3	213	39	85	68	16·6	215	43	90	69	18·0	220	44	18·0	1·57	223	3·65	1·00	,,
June 1	78	62	14·2	167	39	66	63	17·8	87	39	71	69	13·9	146	39	77	69	16·1	227	45	87	65	12·1	219	50	96	71	13·9	188	66	18·6	1·56	375	4·19	0·98	,,
2	76	60	11·4	149	41	75	65	21·5	115	62	79	68	18·2	164	70	66	65	18·3	219	50	84	62	14·5	203	51	98	63	14·8	197	79	18·9	1·54	354	4·06	1·27	,,
3	82	57	10·7	192	75	62	61	19·9	84	44	72	63	12·7	137	54	85	67	9·1	215	83	86	63	17·7	223	63	102	60	15·5	189	90	19·4	1·46	309	4·34	1·63	,,
4	83	55	14·7	168	71	73	62	21·6	93	65	41	60	14·7	153	46	89	66	14·9	211	58											20·1	1·43	386	4·12	1·61	,,
Average of 16 days	74	58	10·9	156	58	66	64	18·6	97	46	68	64	14·5	142	46	75	65	13·9	195	49	81	64·5	13·1	204	59	93	66	14·7	186	78	18·1	1·49	321	3·89	1·37	
Average from 20-24th			9·2					15·3					12·0					11·5					11·5					13·0					274			

May 24: Swimming bath 2.50—5.35 p.m. Rectal T. down from 101·6° to 98·5°. May 27: Exercise stripped and cold 4.10—6.50 p.m. Rectal T. down from 101·8° to 99·8°. May 30: Exercise in heavy clothing 3.10 - 5.50 p.m. Rectal T. up to 103·2°. June 2: In bed till 12.15. Meals as usual. June 3: Swimming bath 2.50 5.30 p.m. Rectal T. down from 101·2° to 98·8°.

TABLE IX

N., C., U., P_2O_5, as in Table VII.

Date.	8.30 A.M.				12.30 P.M.				4.30 P.M.				8.30 P.M.				11.30 P.M.				Total in 24 hours ending at 12.30 p.m.				
1907, Sept.	N.	C.	U.	P_2O_5	N.	C.	U.	P_2O_5	N.	C.	U.	P_2O_5	N.	C.	U.	P_2O_5	N.	C.	U.	P_2O_5	N.	C.	U.	P_2O_5	
2					59	83	31	37	61	82	20	104	65	83	21	147	72	83	29	152			499		[J. B. L.] Diet began Sept. 1. Four meals, at 8.30 a.m., 12.30, 4.30, 8.30 p.m., each of 570 c.c. milk, 170 g. bread, 30 g. butter. = ca. 56 Cal. per kg. and 22 g. N. per diem.
3	78	75	19	112	55	84	19	38	80	85	26	134	76	82	26	181	83	82	21	152	16·5	1·93	499	2·62	
4	73	76	12	105	60	85	28	41	78	86	23	117	75	79	19	155	75	85	23	176	17·7	1·94	492	2·82	
5	79	78	22	124	63	86	31	47	68	88	29	136	77	89	26	157	81	89	23	167	18·0	2·02	539	2·92	
6	78	75	22	117	56	88	25	38	82	84	30	151	88	86	28	166	76	77	24	139	17·5	1·94	589	2·87	2 hrs. 40 mins. at swimming bath till 4 p.m.
7	77	75	23	123	60	82	32	56	70	86	31	147	82	87	26	208	76	86	19	182	18·5	1·96	643	3·02	
8	75	72	18	123	63	83	22	54	75	91	31	146	86	89	27	173	71	87	27	125	17·6	1·98	537	3·29	
9	65	76	22	116	59	88	34	62	73	88	32	157	76	88	30	211	90	85	31	185	16·8	1·99	645	2·94	
10	73	72	23	116	74	82	33	54	53	86	[20]	60	67	96	45	65	69	87	38	167	18·2	1·97	685	3·28	Hard work with two 10 lb. dumb bells 1.30
11	67	80	31	158	68	83	41	77	78	89	32	169	99	89	35	225	110	87	28	198	15·6	2·04	816	2·73	[4.30 p.m.
12	78	78	24	127	64	82	36	87	71	83	31	141	75	83	23	183	85	84	29	187	20·1	1·97	719	3·66	
13	83	78	21	139	70	82	[41]	70	95	88	[47]	177	95	86	[56]	236	101	84	[49]	227	18·7	1·98	659	3·39	173 g. sweetbread at 9 a.m.
14	86	70	[35]	146	71	83	[42]	53	72	82	33	151	75	82	25	155	86	80	[29]	181	21·3	1·88	[1051]	3·86	173 g. sweetbread at 8.30 p.m.
15	92	68	[46]	150	62	86	[54]	63	82	79	[51]	159	85	81	[41]	176	87	78	[41]	174	19·2	1·85	[943]	3·36	
16	76	[74]	24	93	58	86	36	41	72	88	38	146	81	90	33	184	90	87	28	143	18·5	[1·95]	[851]	2·87	
17	77	75	22	121	59	83	28	55	78	86	28	150	72	82	26	166	97	82	21	139	18·1	1·93	672	3·04	
18	84	74	23	128	62	75	26	44	80	83	30	126	84	86	30	190	83	92	25	167	19·0	1·91	589	3·01	
19	77	74	23	115	74		37	59	66		[24]	99	81	89	31	103	82	83	26	168	19·0		673	3·04	Work with 3 lb. dumb-bells 1.30—4.30 p.m.
20	79	75	22	126	74	84	33	66	66	84	28	165	73	84	28	205	94	84	27	214	18·5	1·94	624	2·71	
21	84	76	24	142	81	74	18	63	49	78	14	58	71	86	27	155	70	81	27	201	19·1	1·87	593	3·65	Stayed in bed till 12.30. No food till 12.30
22	75	73	23	143	67	70	34	72	80	83	34	186	95	80	28	169	66	72	22	145	16·4	1·81	585	3·03	
23	67	75	21	128	71	79	36	90	104	86	36	169	107	83	29	206	95	73	19	194	17·8	1·89	643	3·38	0·5 g. Calomel at 11.30 p.m.
24	79	72	17	113	55	85	30	50	60	82	24	140	70	87	28	213	86	87	24	248	20·6	1·93	585	3·30	
25	80	72	20	145	60	81	30	69	87	84	33	188	91	89	35	228	93	79	31	192	17·4	1·91	585	3·64	
26	86	75	25	137	74	84	36	60	63		29	148	93	-	36	281	106		32	178	20·8	—	733	3·72	Three hours in room at 37 C. from 1.30 to 4.30.
27	89	—	26	146	73		34	60	55		26	59	71	86	33	145	80	80	31	189	20·3		725	3·54	In bed till 12.30. No breakfast till 12.30.
28	81	70	27	147	56	76	29	73	56	77	35	118	105	79	47	196	128	80	43	203	17·0	1·79	694	3·00	Bicycling 10 a.m. to 4.30 p.m. No breakfast till
29	79	[64]	24	143	83	—	34	45													20·4		806	3·30	Breakfast at 8.30. In bed till 12 30. [12.30.
Average	78	74	22	127	65	82	32	57	72	84	30	134	82	85	31	180	86	83	27	176	18·3	1·93	652	3·14	

TABLE X

N., U., B., T.P., P_2O_5, H_2O., as in Table VII.

[E. L. K.] Diet began Oct. 30. Three meals daily, at 9 a.m., 2 and 7 p.m., each of 750 c.c. milk and 230 g. bread. = ca. 44 Cal. per kg. and 22 g. N. per diem.

Date	9 A.M.						2 P.M.						7 P.M.						12 P.M.						Total for 24 hours ending at 12 P.M.					
1907.	N.	U.	B.	T.P.	P_2O_5	H_2O	N.	U.	B.	T.P.	P_2O_5	H_2O	N.	U.	B.	T.P.	P_2O_5	H_2O	N.	U.	B.	T.P.	P_2O_5	H_2O	g. N.	mg U.	mg. B.	mg. T.P.	g. P_2O_5	c.c. H_2O
Oct. 31			1·8	6·3			58	19·1	2·8	9·3			61	21·6	3·9	10·2			64	17·2	2·1	7·2			14·3	527	60	190		
Nov. 1	63	12·8		—		35	82	26·4	—	—		125	79	19·9	—			81	62	16·4	-	—		36	16·9	428	-	—		1·52
2	73	15·4	3·4	8·5		37	57	18·5	2·3	8·7		38	125	30·1	4·6	14·7		115	80	18·9	3·1	9·4		42	19·7	476	81	236		1·31
3	79	15·4	3·1	8·2		40	88	20·8	3·7	10·7		78	91	19·2	4·7	11·1		72	102	23·6	2·6	10·4		74	21·1	456	83	235		1·48
4	70	13·6	2·1	6·6		42	78	19·5	3·4	9·9		84	90	18·9	4·0	10·3		90	76	15·1	2·5	7·5		43	18·5	390	68	200		1·46
5	77	13·5	2·2	6·7	149	38	98	23·9	4·3	12·3	66	70	103	21·8	3·5	10·8	162	68	91	19·1	2·9	9·3	219	45	21·3	445	73	222	3·58	1·25
6	85	12·5	2·8	6·9	138	42	100	17·1	3·0	8·7	66	85	99	23·0	3·0	10·7	152	81	90	17·0	2·6	8·3	193	47	22·1	398	68	201	3·29	1·45
7	77	12·8	1·6	5·8	126	39	51	25·8	3·1	11·7	109	75	93	20·2	2·9	7·6	150	47	100	19·2	2·0	8·4	213	44	19·2	441	54	201	3·50	1·18
8	76	15·3	1·8	6·9	131	31	96	27·9	2·1	11·4	89	47	105	23·9	1·9	9·9	144	52	86	17·1	2·6	8·3	194	40	21·2	482	50	211	3·31	0·98
9	82	17·6	2·7	8·6	131	34	41	19·4	8·0	14·5	25	19	109	50·2	5·5	22·3	113	51	113	47·0	4·3	20·0	213	47	20·8	758	109	362	3·03	0·91
10	88	23·7	2·5	10·4	118	33	84	36·4	2·3	14·1	98	37	107	31·4	2·5	13·0	182	46	98	27·7	2·5	11·7	208	41	22·3	689	59	289	3·51	0·92
11	77	19·0	2·2	8·6	122	32	98	34·3	3·4	14·8	59	71	105	26·3	4·5	13·3	172	88	90	15·8	3·3	8·6	198	70	21·6	553	76	260	3·23	1·43
12	48	10·0	3·3	6·6	109	40	100	22·9	6·2	13·9	71	113	101	14·3	6·7	11·4	154	146	110	8·9	7·4	10·3	187	84	19·9	321	131	238	3·05	2·07
13	43	6·2	2·1	4·1	111	41	89	17·2	6·3	12·1	62	127	70	22·3	3·9	11·4	172	93	88	14·8	3·8	8·7	191	57	16·2	326	88	197	3·13	1·75
14	75	8·9	3·7	6·7	114	55	93	26·3	2·5	11·3	66	81	98	22·2	3·0	10·4	163	83	92	10·0	4·4	7·7	237	79	20·8	373	83	207	3·36	1·71
15	66	12·7	2·3	6·5	119	42	88	25·7	4·2	12·8	66	71	99	23·7	4·6	12·5	161	82	87	16·3	4·0	9·5	187	48	19·8	443	86	234	3·15	1·39
16	72	14·0	2·2	6·8	122	37	83	10·2	6·2	9·6	62	201	71	14·8	3·5	7·7	105	80	80	13·3	3·0	7·4	179	52	18·2	318	83	189	2·83	2·02
17	62	12·8	1·9	6·2	121	34	87	26·7	3·2	12·1	75	59	92	25·6	3·2	11·7	178	54	91	18·5	3·6	9·7	188	47	19·1	469	67	224	3·29	1·10
18	79	14·3	3·1	7·9	115	39	95	28·3	3·5	12·9	93	68	101	24·0	3·4	11·4	174	64	83	14·8	2·8	7·7	181	42	21·1	460	76	229	3·23	1·23
19	74	12·5	2·7	6·9	119	40	80	24·0	3·2	11·2	68	49	98	19·0	4·0	10·4	188	77	95	17·0	4·4	10·1	171	58	20·1	415	82	220	3·14	1·27
20	75	11·8	2·9	6·9	111	47	81	10·6	8·5	12·0	59	165	64	11·5	4·3	8·1	93	76	52	7·1	3·5	5·8	106	34	16·6	252	108	191	2·30	1·78
21	69	12·8	3·2	7·5	108	56	79	18·9	2·6	8·9	98	57	83	18·0	2·7	9·0	157	52	87	17·8	2·9	8·8	180	49	18·7	388	70	199	3·15	1·29
22	71	14·0	2·3	7·0	99	37	92	25·7	3·4	12·0	66	81	77	22·5	4·3	11·8	151	80	83	18·3	2·2	8·3	189	44	19·0	459	70	223	2·92	1·36
23	75	14·4	2·0	6·6	117	42	88	23·3	2·7	10·5	62	92	96	13·4	5·4	9·8	120	121	88	12·5	4·3	7·5	167	70	20·4	376	80	205	2·80	1·78
24	58	11·4	1·9	5·7	133	34	72	23·3	2·5	10·3	98	50	100	25·1	3·5	11·8	184	64	90	12·6	3·3	9·2	193	47	18·4	433	64	208	3·57	1·11
25	80	15·0	2·9	7·9	122	42	91	25·9	4·4	13·4	71	89	101	21·7	4·3	11·5	177	97	91	23·1	4·5	12·3	172	85	21·4	494	93	257	3·40	1·73
26	79	36·2	3·0	15·1	189	83	70	38·2	3·3	17·0	72	48	91	27·4	4·0	13·1	160	54	91	16·4	4·6	10·0	150	51	19·7	739	86	332	3·54	1·55
27	75	2·8	3·5	4·5	123	39	76	12·9	2·7	7·0	79	44	90	18·5	4·2	10·3	181	54	90	18·8	2·7	8·9	189	48	19·6	277	82	174	3·35	1·08
28	75	14·5	3·1	8·0	115	53	78	20·2	3·5	10·3	65	60	58	10·2	3·1	6·5	100	54	130	20·1	5·3	12·0	254	107	20·1	383	88	216	3·13	1·58
29	75	8·9	3·5	6·5	123	60	94	11·9	5·6	9·6	73	204	63	13·8	3·9	8·5	107	61	78	12·0	3·3	7·3	151	28	18·3	270	96	186	2·76	2·00
30	64	11·3	1·8	5·5	118	60	83	25·4	3	11·4	69	96	89	21·7	3·8	11·1	174	77	83	18·7	2·9	9·2	184	64	18·5	431	65	208	3·20	1·73
							41	4·8	8·8	10·4	26	11																		
Dec. 1	62	12·5	2·5		119	39	54	32·0	10·1	20·7	26	10	83	48·1	3·7	19·8	53	40												
Average	72	12·8	2·6	6·9	123	43	86	23·3	3·4	11·1	73	82	91	20·7	3·9	10·8	153	74	88	16·6	3·5	9·0	188	54	19·7	429	75	218	3·18	1·45

Average is from 21 normal days. Nov. 7: Walk from 10 a.m. to 2 p.m. Nov. 9: Severe muscular work 10 a.m.–3 p.m., sawing and chopping wood. Nov. 12: 500 c.c. infusion of 3 g. tea with 7 o'clock meal. Nov. 13: 500 c.c. solution of 0·06 g. caffein with 7 o'clock meal. Nov. 14: 500 c.c. solution of 14 g. lactic acid neutralized with soda with 7 o'clock meal. Nov. 16: 9 o'clock meal omitted, 750 c.c. water taken instead; cold day. Nov. 17: 10 mg. Strychnin nitrate at 12 and 5 p.m. Nov. 18: 10 mg. Strychnin nitrate at 9, 12, and 1. Nov. 19: 9 o'clock meal omitted, 750 c.c. water taken instead. Nov. 20: 9 o'clock meal omitted, 750 c.c. water at 9 instead; no meal at 7, one at 12 p.m. instead. Nov. 21: 9·25 g. NaHCO₃ in milk at 7 p.m., 250 c.c. water afterwards. Nov. 22: Urine alkaline till 7 p.m. Nov. 23: Strychnin at 12, 3.30 and 8.30 p.m. Nov. 21. Strychnin at 12 and 3.30 p.m. Nov. 25: 4 g. Salicylic acid neutralized with soda in 250 c.c. water at [illegible] p.m. Nov. 27: 0·03 Pilocarpin nitrate at 9.15 p.m. Nov. 29: Stayed in bed till 1 p.m.; 700 c.c. water at 9. [illegible] in bed till 1 p.m. [illegible] breakfast [illegible] Dec. 1: 700 c.c. water at 9 a.m., no milk or bread. Two 10 lb. dumb-bells raised f[illegible] floor to above [illegible] Nov. [illegible]

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THE PRINCIPLES INVOLVED IN THE STANDARDISATION OF DISINFECTANTS AND THE INFLUENCE OF ORGANIC MATTER UPON GERMICIDAL VALUE.

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CONTENTS.

PAGE
I. Introduction 655
II. Conditions which must be complied with in comparing the germicidal value of disinfectants, and the means suggested to satisfy them . . . 658
  (1) Uniformity in temperature 659
  (2) Uniformity in number of bacteria per unit volume employed . . . 660
  (3) Uniformity in resistance of bacteria employed 661
  (4) Uniformity in culture media 661
  (5) Uniformity in time adopted for the test 662
III. Modification of Rideal-Walker method necessitated by the introduction of a standard time 665
IV. Special considerations involved in the standardisation of disinfectants containing metallic salts 667
  Summary of Chapters II, III and IV 670
V. On the choice of an organism for use in the determination of germicidal values 671
  (1) Variations in resistance of different vegetative forms to three different types of disinfectants 671
  (2) Comparative resistance of spores and vegetative forms to disinfectants . 673
  (3) Differences in resistance to disinfectants between virulent and avirulent strains of the same organism 677
  (4) Summary 677
VI. Influence of organic matter upon the efficiency of disinfectants . . . 677
  (1) Introductory 677
  (2) Soluble organic matter, experiments with blood serum 680
  (3) Particulate organic matter 682
    (a) Animal charcoal 682
    (b) Dust 684
    (c) Finely precipitated coagulated horse serum 685
    (d) Bacteria 685
  (4) Faeces 686
  Summary 689
VII. A method of standardising disinfectants in the presence of faeces . . 690
VIII. Bibliography 695

CHAPTER I. INTRODUCTION.

THE importance of the standardisation of disinfectants is universally admitted but no unanimity of opinion has been arrived at regarding the conditions under which germicidal value should be determined It is the precise conditions under which the observations are to be made, upon which agreement is so necessary, but so difficult to obtain. An agreement is essential for the reason that the efficiency of every different class of disinfectant is differently affected by alteration in the circumstances of its use. Standard conditions for testing purposes have been difficult to define because in practice disinfectants are used as germicides under a variety of conditions, of which the following afford typical instances:—

(1) For the chemical sterilisation of surgical instruments and the hands of the operator, and for various antiseptic lotions and washes, in which case they are mixed with water and are not subjected to the possibly disadvantageous admixture of other substances.

(2) For the purpose of preserving sera, vaccines, etc. against possible bacterial contamination, in which case they act in the presence of a considerable amount of soluble organic matter.

(3) For the disinfection of utensils, soiled linen, closet pans, dejecta, etc. and for the washing of walls and articles of furniture.

By far the greatest quantity of disinfectants is sold for one or other of the purposes detailed under No. 3.

The determination in water of the germicidal value of a preparation will give a serviceable indication if it is to be used for some purposes, but will not necessarily be a reliable guide to its value when mixed, for example, with blood serum or dusty water or the dejecta of a typhoid patient.

It is impracticable to standardise disinfectants under a great variety of circumstances, but it is important to ascertain the effect upon various classes of disinfectants of such conditions as those under which they are commonly employed, and it is likely that the determination of the value of disinfectants in the presence of some definite amount of organic material, both particulate and in solution, will afford a more useful guide to their value from a hygienic point of view.

The work published during the last few years upon the nature of the disinfection process and the laws it obeys (Krönig and Paul (1897), Madsen and Nyman (1907), H. Chick (1908)) has made it possible to formulate the fundamental conditions which must be satisfied in the comparison of germicidal values. These conditions and means suggested for satisfying them are first dealt with in the present paper.

Experiments have been made with three types of disinfectants commonly employed, viz. phenol, mercuric chloride and two emulsified disinfectants[1], referred to as disinfectants "A" and "B." Each of these types has been studied with sporing organisms, *B. subtilis* and *B. anthracis,* with *B. typhosus* and *B. paratyphosus* and with *Staphylococcus aureus* and *B. pestis.* The influence of the virulence or otherwise of the strain of organism has also been considered.

Particular attention has been given to the influence of various forms of organic matter both in suspension and solution upon the germicidal action of these types of disinfectant, experiments being made upon some or all of the above organisms.

The results of our experiments have led us to suggest some modification in the methods commonly employed for the standardisation of disinfectants, and at the end of the paper we put forward a scheme for the standardisation of disinfectants intended for use upon dejecta, or for other purposes involving the presence of a considerable amount of suspended organic matter.

Historical. Systematic experiments comparing the values of different substances to hinder putrefaction were made as long ago as 1750 by *Pringle* (1750). *Buchholtz* (1875) made extensive experiments with a great variety of reputed disinfectants, using an infusion of tobacco leaves, and *Jalan de la Croix* (1881) covered much the same ground, using broth made from meat and the organisms naturally occurring in it. *Baxter* (1875) used vaccine lymph and glanders nodules as test materials, and emphasized the influence of associated organic matter in diminishing the value of the disinfectant.

Robert Koch (1881), however, was the first to compare the germicidal value of various disinfectants upon *pure* cultures of bacteria. He worked with emulsions of anthrax spores dried upon silk threads and estimated the time of survival in solutions of the then known disinfectants, with the result that mercuric chloride has since been justly regarded as the most efficient disinfectant where spores are concerned. Koch's thread method was also used by *Behring* (1890) in a number of experiments in which comparison was made of the germicidal value of a series of disinfectants upon anthrax spores ; Behring also published a number of results of experiments made with many vegetative forms, e.g. anthrax bacilli, *B. cholerae*, *B. typhosus*, streptococci and staphylococci. *Geppert* (1889 and 1891) confirmed Koch's results, but criticised the thread method in view of the unavoidable carrying-over of traces of disinfectant into the test culture, these traces preventing growth of the organisms adherent to the thread, although they might not have lost their vitality at the moment of subculture. On adding to the test culture sufficient ammonium sulphide to precipitate the traces of mercuric chloride that were thus carried over, when working directly with emulsions of bacteria in place of the silk threads soaked in broth cultures, Geppert found that the organisms might still grow[1], and concluded that the germicidal value of mercuric chloride, though doubtless preeminent where spores are concerned, had been overestimated. *Gruber* (1891) advocated working with emulsions of bacteria and supported Geppert's view as regards the overcarriage of $HgCl_2$, the misleading results of which he avoided by successive dilution of the test sample.

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[1] The active principles in these emulsified disinfectants were derivatives of higher tar acids; the greater part of these acids distilled over between 210 and 300° C. Disinfectant A contained glue, and Disinfectant B resin and soft soap as emulsifiers. Both A and B formed extremely fine emulsions when diluted with distilled water.

About the same time Creolin, the first of many emulsified disinfectants, received a great deal of attention from many workers ; its germicidal value was compared with that of the older disinfectants, various organisms and different methods being used. *Esmarch* (1887) found it to be far superior to carbolic acid for the disinfection of emulsions of *B. cholerae* and streptococci, and less efficient for disinfection of anthrax spores, using the thread method. *Henle* (1889), also working with emulsions of bacteria, obtained excellent results with *B. typhosus*, while *Fraenkel* (1889), working with anthrax spores and the thread method, demonstrated the high disinfecting power of cresols and higher phenols, to which the germicidal value of creolin as well as that of crude carbolic acid is due.

Krönig and Paul (1897) devised a method for estimating germicidal value of disinfectants with anthrax spores, which had none of the drawbacks of Koch's thread method. Garnets were selected of similar size, carefully cleaned and dipped into an emulsion of sporing anthrax bacilli which was allowed to dry on their surface in a thin film. The garnets were then immersed in the solution of the disinfectant in question ; from time to time a definite quantity was taken out, the disinfectant carried over was removed by gentle washing with water and, if necessary, with a precipitant (e.g. ammonium sulphide in the case of mercuric chloride). The garnets were then vigorously shaken in a measured quantity of water to detach the adherent spores and a constant amount of the washings was plated. The number of germinating organisms was counted, it having been shown that by vigorous shaking in water a fairly constant proportion of the total organisms was detached. By this method comparison could be made of the velocity of disinfection in the case of different disinfectants under different conditions. Krönig and Paul published a large number of very careful experiments, made with disinfectants of every class and in widely varying concentrations. They were the first to realize that the relative value of disinfectants depends very largely upon the conditions under which they work, and laid down the general laws that in any comparison of disinfectants close regard must be paid to the following conditions :

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[1] The effect of treatment with a sulphide is not entirely due to the neutralisation of the $HgCl_2$ carried over in solution (*vide* p. 668).

(1) Constancy of number and species of bacterium used.
(2) Constancy of temperature.
(3) Constancy of nutrient medium for test cultures.
(4) Absence of other organic matter during disinfection.

They scrupulously observed all of these conditions, thus making their very exhaustive set of experiments the most valuable work which has yet appeared upon the subject of disinfection. A full description of the method as applied to the practical determination of the germicidal value of disinfectants was afterwards published by *Paul* (1901), anthrax spores and mercuric chloride being recommended as standard organism and disinfectant respectively.

Rideal and Walker (1903) published a method for the standardisation of disinfectants. They also realized the importance of the conditions of experiment. In their method (known as the "drop" method) a definite small amount of a broth culture of constant species and age was added to a constant volume of disinfectant solution. *B. typhosus* was chosen as the standard organism, and pure phenol as the standard disinfectant. By a series of trials with different concentrations both of disinfectant and pure phenol, under otherwise similar conditions, these authors determined the relative concentrations necessary to complete germicidal action in the same time. The ratio of the reciprocals of these concentrations was called the "carbolic acid coefficient" and was taken to express the germicidal value of the disinfectant in terms of carbolic acid as a standard.

This method has come into very general use, and been widely commended, among others by *Firth and Macfadyen* (1906) who found it to be superior in accuracy and convenience both to a method based on the "garnet" method of *Krönig and Paul* (1897) and the "thread" method of *Robert Koch* (1881).

The method of Rideal and Walker, in common with all preceding methods of standardisation, has been considered, in the opinion of many, to suffer from the disadvantage that whereas, in the majority of cases of practical disinfection, organic matter of some sort is present, there is no attempt to realize this condition during standardisation. Accordingly Kenwood and Hewlett (1906) and Wynter Blyth (1906) have suggested modifications of the Rideal and Walker method in which organic matter of different kinds is introduced; these modifications will be described later, in the section dealing with the effect of the presence of organic matter upon the efficiency of disinfectants.

CHAPTER II. CONDITIONS WHICH MUST BE COMPLIED WITH IN COMPARING THE GERMICIDAL VALUE OF DISINFECTANTS, AND THE MEANS SUGGESTED TO SATISFY THEM.

Disinfection has been shown by Madsen and Nyman (1907) and H. Chick (1908) to be a process exhibiting many analogies with a chemical reaction, one reagent being represented by the bacterium and the second by the disinfectant. When the disinfectant is present in considerable excess the process proceeds in accordance with a definite law, the number of living bacteria per unit volume progressively and regularly decreasing with increase of time in a logarithmic ratio. This law is expressed by the equation

$$-\frac{dn}{dt}=Kn,$$

or

$$\frac{1}{t_2-t_1}\log\frac{n_1}{n_2}=K,$$

where n_1 and n_2 are the numbers of bacteria surviving in unit volume after times t_1 and t_2 respectively.

In determining the relative germicidal value of disinfectants, whatever the procedure adopted, the temperature at which the disinfection takes place and the number in unit volume and resistance of the bacteria employed for the test must, as was pointed out by Krönig and Paul (1897, p. 3), be constant. If an end-point method is used, such as that of Rideal and Walker, the nutrient medium into which the test samples are withdrawn must not be altered during the progress of the experiment, and it is also necessary to maintain uniformity in the time allowed for the disinfectant to act.

The reasons which make constancy in these several points a necessity are discussed below.

1. *Temperature.* The velocity of disinfection has been shown to be influenced by temperature in accordance with the equation of Arrhenius. In the case of mercuric chloride and anthrax spores disinfection velocity was found to be increased 3 times for every 10° C. rise in temperature (Madsen and Nyman, 1907); in the case of disinfection of *B. paratyphosus* with metallic salts it was influenced to the same extent, but with phenol and emulsified disinfectants the velocity was increased 7—8 fold for every 10° C. rise in temperature (H. C. 1908, pp. 146 and 148). In consequence it is necessary that during any determination of germicidal value standard and disinfectant should be maintained at the same temperature, and germicidal values cannot be directly compared unless the temperature at which the determination was made is the same.

The general effect of rise in temperature in assisting disinfection has been known for some time (Koch (1881), Henle (1889), Behring (1890), Heider (1892)), and the necessity of keeping temperature constant during standardisation was insisted upon by Krönig and Paul (1897) and by Paul (1902). In our own work 20° C. was fixed as a convenient temperature, and one approximating to the conditions of practical disinfection, and all experiments were made in a bath kept within half a degree of this temperature.

2. *Number of bacteria.* It follows from the law of disinfection formulated above that even when the disinfectant is present in great excess the greater the number of bacteria present in a unit volume the longer will be the time required for their disinfection. This effect may be seen from the instances in Table I below.

TABLE I.

Showing the effect upon the time taken for disinfection by phenol of varying the number of bacteria per unit volume.

B. paratyphosus 21° C.

Concentration of phenol parts per 1000	No. of organisms in unit volume	Time taken for disinfection, mins.
10	440,000	0·75
10	66,000,000	6·0
8	187,000	2·25
8	440,000	4·5
8	56,000,000	32·75
8	66,000,000	34·5
6	110,000	17·5
6	16,000,000	141

To obtain a constant result with a particular disinfectant, it is essential to work always with the same concentration of organisms, but in comparative experiments this is not necessary as long as they are consistent. Therefore, if the comparison of germicidal value is always made against a standard, the standard being tested at the same time against the same amount of culture, no inaccuracy results, even if the number of organisms should vary from time to time. Such variations are, however, extremely inconvenient, as they make it difficult to arrange beforehand experiments to yield the necessary negative and positive results.

A broth culture containing about 250 to 500 millions of *B. typhosus* per c.c. was obtained by sowing 6 c.c. of broth with a loopful from a fresh agar culture, and incubating for 24 hours at 37° C. Such a seeding is an excessive one, so that although the number of bacilli sown may vary considerably, there are always enough added to obtain within 24 hours the maximum growth the medium can sustain.

3. *Resistance of the culture.* Any variation in the resistance of the culture although not introducing any error in experiments where disinfectant and standard are simultaneously tested is also inconvenient for the reason discussed in the preceding paragraph. It is therefore very important to obtain cultures of a standard resistance.

The difference in resistance between a culture freshly isolated and one grown for some time upon culture media will be referred to later (p. 677), but even in the case of a stock laboratory culture of *B. typhosus*, it was found that its resistance to phenol was considerably modified by its previous history. If such a stock culture were successively subcultured and maintained at 37° C., its resistance to disinfection was found to progressively increase; when kept in the cold room, after growth had taken place at room temperature, its resistance was found to be gradually lessened although in both cases the culture actually used for the experiment had been grown at 37° C.[1] It was found most satisfactory to grow and maintain the stock culture on sloped agar at room temperature (16—20° C.), subculturing about every two weeks. From such growths a standard loopful was sown in broth and cultivated for 24 hours at 37° C. By these means cultures of moderately constant resistance were obtained.

4. *Culture media.* It was found convenient, though not indispensable for accuracy in a comparative experiment (see above), to maintain the culture medium as constant as possible.

The exact nature of the medium employed for the test cultures, though not affecting the coefficient if both disinfectant and standard were simultaneously tested, was found to influence very much the actual result obtained. If as is the case with end-point methods, few, and possibly somewhat damaged, organisms be transferred to the culture tubes, the chances of growth are considerably greater in a more favourable medium. Thus the germicidal value of any disinfectant will apparently vary with variation in the medium employed for the test cultures, although the value relative to that of the standard phenol will remain constant. This is well shown in Table II.

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[1] Rideal and Walker (1903, p. 431) concluded that increased temperature of incubation raised the resistance of *B. typhosus* to disinfectants. The data given are, however, insufficient, as no mention is made of the number of organisms, per unit volume being the same in the two experiments cited.

To obviate the variation due to unavoidable differences in beef broth, Brand's "Meat Juice" was substituted for fresh beef broth, and the following medium was adopted:

Brand's Meat Juice	10 c.c.	in 1 litre tap-water.
Salt	5 gr.	
Peptone	10 gr.	
Glucose	10 gr.	

The medium had a reaction of + 6 to + 7 to phenolphthalein according to the notation of Eyre.

A large stock of the "Meat Juice" was put up in quantities of 10 c.c. and sterilised. The medium made with this preparation is not quite as favourable to the growth of *B. typhosus* as that made with fresh beef extract, but has been found to work well in practice.

TABLE II.

Influence of the suitability of the culture medium into which test samples are seeded upon the apparent germicidal value. The experiments were made with B. typhosus and all other conditions were identical.

			Concentration required to kill in 15 minutes		
	Disinfectant	Medium	Disinfectant parts per 1000	Pure phenol parts per 1000	Carbolic acid coefficient of the disinfectant
Exp. 23. 1. 08	I	Broth A	4·4	10·5	2·4
,, 31. 1. 08	,,	Broth B	5·2	12·7	2·4
,, 23. 1. 08	II	Broth A	3·5	10·5	3·0
	,,	Broth B	4·4	12·7	2·9
,, 17. 1. 08	III	Broth A	8	10·5	1·3
	,,	Broth B	10	13	1·3
,, 23. 1. 08	IV	Broth A	3·5	10·5	3·0
,, 7. 2. 08	,,	Broth B	4·8	13	2·7

5. *Time.* It has been shown by one of us (H. C. 1908) that a logarithmic relation exists between concentration of disinfectant and velocity of disinfection. This was found to be true in the case of such different organisms as *B. paratyphosus*, *Staphylococcus pyogenes aureus* and spores of *B. anthracis*, and such disinfectants as phenol, an emulsified disinfectant containing higher coal tar derivatives, and mercuric chloride (when the concentration of the metallic ions is taken into account). It follows that variations in concentration of disinfectant correspond to greater differences in the time taken for disinfection.

It was also found that the effect upon the time taken to kill of varying concentration is not the same with different classes of disinfectants. Two concentrations of two disinfectants, which are equally efficient, if allowed to operate for 5 minutes, do not necessarily bear the same relationship to one another as do two other concentrations of the same two disinfectants, which are equally efficient, if the time chosen for the test is 20 minutes. It follows that in comparing germicidal value in two cases where the effect of varying concentration upon the velocity of disinfection is very different (e.g. metallic salts and phenoloid bodies) completely different phenol coefficients will be obtained according to the time taken for the test, see Table III. If however, in comparing the germicidal value of two disinfectants, they are such, that the effect of altering concentration upon the time taken for disinfection is about the same, it does not signify very much what time is taken for the test. An example of this is seen in the comparison of germicidal values of phenol and emulsified disinfectant "A" towards *B. paratyphosus* (see Table IV). On reference to a previous paper by one of us (H. C. 1908, pp. 119 and 122) it will be seen that the curves obtained by plotting concentration against time of disinfection are very similar for phenol and the coal tar disinfectant "A," and in the table

TABLE III.

Showing the carbolic acid coefficients of mercuric chloride and silver nitrate, with variation in the time during which the disinfectant is allowed to act.

B. paratyphosus 20° C.

Disinfectant	Time of disinfection minutes	Concentration of disinfectant grm. per 1000	Carbolic acid coefficient
Mercuric chloride	2·5	0·88	13·6
Phenol	2·5	12	
Mercuric chloride	10	0·06	173
Phenol	10	10·4	
Mercuric chloride	30	0·018	550
Phenol	30	9·9	
Silver nitrate	2·5	0·068	176
Phenol	2·5	12	
Silver nitrate	10	0·015	693
Phenol	10	10·4	
Silver nitrate	50	0·009	922
Phenol	50	8·3	

below (Table IV) the coefficients obtained by comparing the respective concentrations are the same within the error of such experiments.

TABLE IV.

Showing the constancy in the value of the carbolic acid coefficient of disinfectant 'A' with variation in the time during which the disinfectant is allowed to act.

B. paratyphosus 20° C.

Disinfectant	Time of disinfection minutes	Concentration of disinfectant per 1000	Carbolic acid coefficient of disinfectant 'A'
'A'	2·5	0·8	15
Phenol		12	
'A'	12·5	0·6	17
Phenol		10·2	
'A'	40	0·5	16
Phenol		8·5	

It is obligatory, however, in any method for the standardisation of disinfectants, which shall be applicable to germicides of every class, to choose an arbitrary time, during which the disinfectant shall be allowed to act. What time shall be employed must be decided by considerations of hygiene, and convenience in working the test. Provided these conditions be satisfied, the time chosen should be such that the test be not unduly prejudicial to any particular class of disinfectant. As previously shown, as far as the relationship between the germicidal efficiency of phenol and the emulsified disinfectants containing higher tar acids is concerned, it is immaterial whether the comparison be made after 10 minutes or 30 minutes, but the shorter time is greatly to the disadvantage of mercury and silver salts. We are of the opinion that for practical purposes it is desirable that a disinfection be completed in about half an hour, and this time is quite a convenient one for the laboratory tests. The employment of a shorter interval for the tests would be too unfavourable to metallic salts such as those of mercury and silver, which are undoubtedly amongst the most powerful germicides we possess.

Metallic salts are effective in very small concentration (1000 times less than phenol) and have the peculiarity that, if the concentration is increased, although the velocity of disinfection is increased also, this does not occur to nearly the same extent as with other disinfectants (H. C. 1908, Tables XVII, XIX and XXII). In the case of $HgCl_2$ this is partly but not altogether[1] accounted for by the fact that the Hg^{++} ions are the real disinfecting agent and owing to peculiarities in the ionisation of mercuric salts, changes in concentration of the salts influence the concentration of the ions comparatively little.

Compared with phenol, the action of $HgCl_2$ upon bacteria is slow. For example, it was found that *B. paratyphosus* (6,000,000 per c.c.) could withstand the action of 5 °/₀ $HgCl_2$ for more than four minutes (H. C. 1908, p. 126), and *Staphylococcus pyogenes aureus* in similar concentration for more than 15 minutes (see Table VI, below) if immediately treated with a sulphide solution. This appears to be due to delay in $HgCl_2$ actually "getting to work" upon the contents of the bacterium, and will be discussed later.

Activity in high dilution is a valuable characteristic in a disinfectant. From this point of view mercury and silver are preeminent amongst chemical disinfectants, and it is advisable to choose a time for the standardisation which shall to some extent measure this property. When we embarked upon this enquiry we did not realise to what extent the metallic salts would suffer by comparison when short times were allowed for disinfection, and for most of our work 15 minutes was the time during which the disinfectant was allowed to act. We think however that, for the consideration discussed above, the time should be extended to 30 minutes.

CHAPTER III. MODIFICATION OF RIDEAL-WALKER METHOD NECESSITATED BY ADOPTING A CONSTANT TIME.

The introduction of an arbitrary time, which is necessitated by the considerations dealt with above, involves a modification in the method recommended by Rideal and Walker; unfortunately this modification makes the determination more tedious to perform.

The procedure we adopt is as follows:

Everything used in the experiment, tubes, pipettes, etc., being previously sterilised, a series of tubes containing 5 c.c. of the disinfectant in different concentrations are placed in a water bath at 20° C. When the tubes have taken the temperature of the bath, they are one after another inoculated with five drops of 24 hours' culture of *B. typhosus* from a standard pipette[2], the time being registered by a chronograph.

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[1] Watson (1908). Constant K for $HgCl_2$, when Hg^{++} ions are reckoned as disinfectant, $=3{\cdot}8$, for phenol $K=5{\cdot}5$.

[2] H. C. 1908, p. 96.

Exactly one minute is allowed to pass between each inoculation. When 30 minutes have elapsed since the first tube was inoculated, samples in duplicate are taken from it with a platinum loop[1], and sown in 10 c.c. glucose broth containing litmus (see p. 662). One minute later the second tube is sampled and so on. These test cultures are incubated at 37° C. and always kept four days under observation.

Supposing the value of the disinfectant to be tested is totally unknown, the first series of observations must be scattered over a wide range, e.g. concentrations from 1 in 10 to 1 in 10,000. Having ascertained that the concentration necessary to kill in 30 minutes is between, say, 10 in 1000 and 1 in 1000 the second series is arranged to narrow it down to between, say, 4 and 5 per 1000, and a third series may determine the necessary concentration as between 4·2 and 4·5 per 1000. At this last trial a series of tubes, containing various strengths of pure phenol, are simultaneously tested.

The Rideal-Walker method, when employed to test disinfectants containing the higher coal tar derivatives, has been criticised (Gruber, (1891), Proskauer (1907), Seligmann (1907)), in view of the carrying-over of traces of disinfectant with the test samples, which may cause inhibition of growth in the test cultures. When disinfectants containing metallic salts are in question this carrying-over is of great importance and the traces thus added to the test cultures have to be neutralised. This is conveniently done by the addition of sulphides (see below, p. 668 where the subject is treated in detail). In the case of phenol and coal tar disinfectants there is, however, no suitable neutralising agent, but special experiments made for the purpose showed that there was no error introduced in carrying-over small quantities of these disinfectants. For example, a concentration of 1 in 1000 phenol in the test culture did not inhibit growth either of 10,000 or of 40 organisms in 10 c.c. In the case of disinfectant "B" a concentration of 1 in 10,000 was necessary to cause inhibition of the same concentration of organisms; 1 in 100,000 caused no inhibition in either case (see Table V below). These concentrations are higher than any attained in the test cultures in the course of a standardisation experiment.

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[1] A platinum loop of standard size was used for sampling. It is important to make the sample as large as possible to avoid errors in sampling, consequently the loop was bent horizontally and removed parallel to the surface of the liquid; it was found that the sample was much larger than when the plane of the loop was removed at right angles to the surface of the liquid. According as the sample taken is large or small, the determination is concerned with more or less complete disinfection. The two loopfuls removed (= about 0·0086 gr.) constitute about $\frac{1}{300}$ of the total volume, 5 c.c., and, if the test sample shows no growth, this indicates that the original number, about 30 millions, is reduced to less than 300 (cf. H. C. 1908, p. 118, where a larger sample was withdrawn (0·08 c.c.) and no growth indicated that about 30,000,000 per 5 c.c. had been reduced to less than 60 per 5 c.c.). For practical purposes disinfection may be regarded as complete in either case.

CHAPTER IV. SPECIAL CONSIDERATIONS INVOLVED IN THE CASE OF DISINFECTANTS CONTAINING MERCURY.

In the standardisation of disinfectants containing mercuric chloride and some other salts of heavy metals some complications are encountered which are not met with in the case of the organic disinfectants. Disinfection by salts of mercury exhibits the following two peculiarities.

1. *Mercuric chloride is capable of acting as a germicide even in high dilution* (1 *in* 1,000,000) *if sufficient time be allowed for the action.*

Robert Koch (1881) found that 3 in 1,000,000 mercuric chloride inhibited growth of anthrax spores; Geppert (according to Behring) found that a concentration of 1 in 2,000,000 was sufficient to inhibit growth of anthrax spores; Behring himself found that 1 in 400,000 mercuric chloride was needed to prevent growth of anthrax spores.

TABLE V.

Showing the comparative inhibiting power of disinfectants upon the growth of some organisms.

Date of exp.	Test organisms	Disinfectant	Concentration inhibiting growth, parts per million	Concentration not inhibiting growth, parts per million	No. of organisms sown in 10 c.c.
15. 1. 07	*B. paratyphosus*	Mercuric chloride	1	0·1	1020
30. 6. 08	,,	,,	—	1	5000
28. 5. 08	*Staphylococcus pyogenes aureus*	,,	34	17	5000
			34		3,000,000 to 4,000,000
28. 5. 08	*B. pestis*	,,	1·7	—	2000
15. 3. 07	*B. paratyphosus*	Silver nitrate	1·4	0·14	40 to 80
20. 7. 08	*B. typhosus*	Phenol	—	1000	40 or 10,000
20. 7. 08	,,	Disinfectant B	100	10	40 or 10,000

We have made some determinations of these amounts with different test organisms and a few results with different disinfectants are arranged in Table V, for purposes of easy comparison. The results with mercuric chloride are roughly in accord with those of Koch and Geppert, and also show the influence of the quantity of bacteria introduced upon the result. This difference may be due to selection; the more bacteria introduced, the greater will be the number of specially robust individuals present. Or, there may be an exhaustion of the mercuric chloride owing to the number introduced. Possibly the result may be due to a combination of both effects.

In consequence of this germicidal action in high dilution it is necessary, as originally insisted upon by Geppert, to precipitate the mercury carried over with samples withdrawn during an experiment, as this would otherwise prevent growth in the test cultures. A sulphide is the common precipitant employed and it has been found necessary to add an excess over and above the amount calculated for combination with the mercury carried over (H. C. 1908, p. 123, Tab. XX). The reason for this excess will be discussed when dealing with a second peculiarity of disinfection by mercuric salts. This treatment with sulphide means a little extra trouble, for such precipitants have themselves an inhibiting action upon the growth of bacteria, and it is important not to add enough to interfere with subsequent growth. A saturated solution of hydrogen sulphide[1] in distilled water was found to be the most satisfactory precipitant and 0·2 c.c. added to 10 c.c. of broth (see H. C. 1908, pp. 123—125) was found ample for neutralisation and not sufficient to interfere with subsequent growth.

2. *Organisms submitted to the action of mercuric salts and subsequently washed free of the disinfectant, may not grow if planted directly into broth, but are not necessarily damaged irretrievably, and if treated with a sulphide solution, a certain proportion can be resuscitated.*

These facts have already been recorded by one of us (H. C. 1908, p. 132), and it was suggested that they were due to the formation of a mercury addition-compound with the substance of the bacterium, which prevented the organism manifesting its vitality by growth, until it was decomposed by the action of a sulphide.

The following series of experiments demonstrates the correctness of this interpretation.

To an emulsion of *Staphylococcus aureus* sufficient $HgCl_2$ was added to make a concentration of 1 %. The mixture was immediately decanted into a series of tubes and placed in a rapidly rotating centrifuge ($t = 18 - 20^\circ$ C.). After 13 minutes the supernatant fluid was removed from the tubes and the deposited bacteria thoroughly washed with distilled water in the centrifuge. The water was withdrawn and the tube filled with nutrient broth and incubated. In no case was growth observed. In a second tube the bacteria, after removal of the mercury solution, were at once treated with 1/10th saturated H_2S water. After ten minutes the sulphide solution was removed and the tube filled with broth; on incubation growth occurred. In the case of five other tubes, varying intervals up to five hours elapsed before the sulphide was added, with the result that the addition of sulphide was found to be effective up to 77 minutes after the treatment with mercuric chloride. The addition of the sulphide blackened the washed bacterial deposit, showing that some mercuric salt had been absorbed by the bacterium and formed a compound with its substance. With the mercury attached, subsequent manifestation of life by multiplication is in abeyance unless a large excess of sulphide be applied as antidote.

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[1] The H_2S solution must be fresh.

From the above observations it follows that in determining the germicidal value of $HgCl_2$ (and the same holds for preparations containing salts of the heavy metals) we are confronted with the difficulty of deciding when the bacterium is to be considered for practical purposes dead[1]. Bacteria which have been subjected to the action of dilute solutions of $HgCl_2$ will not multiply unless they come into contact with SH_2 or soluble sulphides. When used for surgical purposes, where there is little chance that the action of the $HgCl_2$ may be reversed by contact with sulphides, mercuric chloride will prove effective even in very great dilution. Should, however, the disinfected material subsequently come in contact with decomposing organic matter, animal dejecta, etc., mercuric chloride will prove less efficient.

It is impossible to institute an accurate comparison between the value of metallic salts and organic disinfectants of the type of phenol. If, in estimating the germicidal value of mercury or silver salts, we omit to treat the sample of liquid containing the bacteria with a sulphide at the expiration of the time allowed for disinfection, we shall form a greatly exaggerated conception of their efficiency, whereas their value will be somewhat underestimated if this procedure be adopted. The latter method is now commonly employed. This escapes the grosser error but affords a severe judgement upon the value of metallic salts when used under certain conditions.

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[1] Most divergent results have been obtained with mercuric chloride. R. Koch (1881), who was the first to lay stress upon the powerful germicidal value of mercuric chloride, obtained an exaggerated idea of its worth because he employed no precipitant to neutralise the traces of sublimate carried over into his test cultures. Geppert (1889 and 1891) pointed this out, but it is probable that he also overestimated the germicidal properties of $HgCl_2$ because, although he neutralised the traces of sublimate carried over, he did not employ a sufficient excess of sulphide.

Summary of Chapters II, III, and IV.

1. In any method of standardisation it is necessary that the test shall be carried out at a constant temperature, as the disinfection process has a high temperature coefficient. For any method to be of general application, it is also necessary that the temperature selected shall be adhered to in all determinations, since the temperature coefficient of disinfection varies for different disinfectants. The temperature adopted was 20° C.

2. To avoid unnecessary labour, the number per unit volume and resistance of the bacteria employed for the test should be kept as constant as possible, and the culture medium used both for the original culture and for the test subcultures should be maintained of constant composition.

Neglect of these precautions does not affect the value found for the carbolic acid coefficient, if disinfectant and phenol are simultaneously tested, but leads to much trouble and inconvenience in the practice of standardisation.

3. It is necessary to arbitrarily select a fixed time during which the disinfectants shall be allowed to act.

A logarithmic relation exists between concentration of disinfectant and velocity of disinfection, and the effect of varying concentration upon the time taken to kill, is different for different disinfectants. Different phenol coefficients may be obtained according to the time adopted in the test.

The time suggested is 30 minutes.

4. In the case of metallic salts, a sulphide must be employed to neutralise the traces of disinfectant carried over with the test sample. With other disinfectants neutralisation was found to be unnecessary.

5. In the case of mercuric chloride, it was found necessary to add a large excess of sulphide over and above that required for combination with the mercury carried over, in order to decompose a compound formed between the mercuric salt and the substance of the bacterium. The presence of this compound prevents manifestation of vitality on the part of the organism unless an excess of soluble sulphide be administered as an antidote.

CHAPTER V. CHOICE OF TEST ORGANISM.

An important advance, made by Koch (1881) upon the method of his predecessors, Pringle (1750), Baxter (1875), L. Buchholtz (1875), and Jalan de la Croix (1881), was the use of pure cultures of bacteria for the determination of germicidal values of disinfectants instead of mixtures of putrefactive bacteria. He employed the spores of *B. anthracis.* Spores are much more resistant to disinfection than most vegetative forms, and many disinfectants which may usefully be employed for the latter are practically valueless against the former, so that Koch's test demanded a high standard of efficiency.

The question arises whether, for practical purposes, such a high standard is necessary. Rideal and Walker's method, which is largely used in this country, and which adopts carbolic acid and *B. typhosus* as standard disinfectant and test organism respectively, assumes that this question may be answered in the negative.

At the time when Koch made his experiments the number of pathogenic organisms, which formed spores, was overestimated. At the present time it is known that the germs of the great majority of diseases to which man is liable are produced by vegetative forms. It must however be remembered that there are a number of diseases about the etiology of which we have no data (e.g. typhus, scarlet fever, measles, etc.) and also several others, common to man and animals, which are known to be caused by spore-bearing organisms (e.g. tetanus, anthrax, malignant oedema, etc.). A disinfectant may at any time be required to obviate risk of infection from any of such diseases, and the departure from the high standard of Koch (mercuric chloride and anthrax as standard disinfectant and test organism respectively) and the adoption of a low standard, such as that of Rideal and Walker (carbolic acid and *B. typhosus*) may give rise to a false sense of security unless it is clearly understood what has been done.

Variation in the resistance of different vegetative forms to three different types of disinfectant.

The types of organisms chosen were (1) *B. typhosus* and *B. paratyphosus*, (2) *Staphylococcus pyogenes aureus*, (3) *B. pestis.* In each case 5 drops (·086 c.c.) from a standard pipette[1] taken from a 24 hours' culture at 37° C. (30° in the case of *B. pestis*) were added to 5 c.c. disinfectant solution.

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[1] See H. Chick (1908, p. 96).

The disinfectants used were phenol, emulsified disinfectant B, mercuric chloride, and oxychinoline sulphonate of potassium ("chinosol").

In each case determination was made of the concentration of disinfectant required to kill the amount of culture added in 15 minutes. The details of the method, which is the one ultimately adopted for the standardisation of disinfectants, has been described in detail (p. 666); it will be sufficient here to give the results obtained (Table VI).

TABLE VI.

	Test organisms	Concentration, parts per 1000, required for disinfection in 15 minutes					
		Pure phenol	Disinfectant B	Relative conc. of Hg^{++} ions	Mercuric chloride[1]	Oxychinoline sulphonate of potassium	Carbolic acid coefficient
Exp. 20. 3. 08	*B. typhosus*	7·9	0·51	—	—	—	15·5
,, 11. 3. 08	,,	8·2	0·54	—	—	—	15·2
,, 22. 5. 08	*Staphylococcus pyogenes aureus*	9·0	2·0	—	—	—	4·5
,, 28. 5. 08	,, ,,	10·5	2·4	—	—	—	4·4
,, 19. 5. 08	*B. pestis*	8·5	0·2	—	—	—	40
,, 3. 6. 08	,,	7·25	0·18	—	—	—	40·3
,, 14.11.07	*B. paratyphosus*	8·00	—	about 42·5	about 0·1	—	about 80
,, 21. 5. 08	*B. typhosus*	7·75	—	46·0	0·15	—	81
,, 2. 6. 08	*Staphylococcus pyogenes aureus*	10·5	—	more than 107·5	more than 50		less than 0·21
,, 2. 6. 08	,, ,,	10·5	—	do.	do.	—	,, 0·21
,, 16. 6. 08	,, ,,	10·5	—	do.	do.	—	,, 0·21
,, 16. 6. 08	,, ,,	9·5	—	do.	do.	—	,, 0·19
,, 3. 6. 08	*B. pestis*	7·25	—	33·5	0·035	—	207
,, 22. 8. 07	*B. typhosus*	6·5	—	—	—	26	0·25
,, 23. 9. 07	*Staphylococcus pyogenes aureus*	9·0	—	—	—	10	0·9

It is very noticeable that, whereas the strengths of carbolic acid required to kill the three types of organism chosen varies comparatively little (see column 2, Table VI), *B. pestis* to a large degree and *B. typhosus* to a less degree show themselves more susceptible to the action both of emulsified disinfectant "B" and mercuric chloride. In the case of "chinosol," the reverse is true, *Staphylococcus aureus* being found more susceptible than *B. typhosus* to the disinfectant.

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[1] H_2S was used for neutralising traces of $HgCl_2$ carried over with the sample into the test culture at the end of the 15 minutes.

The determinations of concentration of $HgCl_2$ necessary for disinfection in 15 minutes (Table VI column 5), and therefore its carbolic acid coefficients are merely approximate. This is owing to the fact that it is not the concentration of $HgCl_2$ as such which is operative, but the concentration of mercuric ions (Krönig and Paul (1897), H. Chick (1908)). The ionisation of mercuric salts is peculiar and 100 °/₀ increase in concentration of the salt, e.g. from 1 to 2 parts per 10,000, only increases the concentration of ions by 15 °/₀, an amount which does not greatly exceed the experimental error of the determination. The numbers representing the concentration of mercuric ions corresponding to various concentrations of mercuric salt, given in Table VI, were obtained from a curve constructed by means of the results of Luther (1904) and Kahlenberg (1901).

Whereas very low concentrations of mercuric chloride are effective against *B. typhosus*, *B. paratyphosus* or *B. pestis* (see Table VI), with the method employed, viz.—treatment with H_2S at the end of 15 minutes—it was found impossible to kill *Staphylococcus pyogenes aureus* by treatment with 5 °/₀ $HgCl_2$ for this length of time. 5 °/₀ of mercuric chloride is a nearly saturated solution, so that a determination of the carbolic acid coefficient of mercuric chloride could not be made.

Comparative resistance to disinfectants of spores and vegetative forms.

It is notorious that spores are much less readily destroyed by disinfectants than vegetative forms, and we have tried to ascertain the relation between the strengths of some disinfectants necessary for the destruction of one and the other.

In order to compare by direct experiment the relative strengths of any given disinfectant required to kill (*a*) spores and (*b*) vegetative forms it would be necessary that approximately the *same number per unit volume* of individuals of either class should be killed in the same time. The relative strengths of disinfectant in the two cases would then be an indication of the relative resistances of the particular spore and vegetative form. It would however be extremely difficult to make such an experiment, the necessary conditions could only be obtained by accident and this never has occurred in the course of an extensive series of experiments. The result could however be calculated from the relation which has been shown to exist between the concentration of disinfectant and time taken for disinfection. Unfortunately we have no available data of our own except in the case of phenol.

Experiments with phenol. B. paratyphosus and anthrax spores. Experiments have been published by one of us in which anthrax spores were destroyed by phenol, and the progress of the reaction was studied by enumerating the spores surviving in unit volume at successive intervals of time. In one such experiment with 5 % phenol at 20° C. (H. C. 1908, p. 97) it was found that 434 anthrax spores in unit volume (a standard drop) of the solution were reduced to 28 in 25·5 hours.

Exactly similar experiments were made with phenol and *B. paratyphosus*, at the same temperature. In one such experiment with 0·6 % phenol it was found that 484 bacteria in unit volume were reduced to 28·5 in 4 minutes (H. C. 1908, p. 106); in a second similar experiment 484 were reduced to 24 in 4·5 minutes (p. 111). It is therefore not very inaccurate to say that in the case of *B. paratyphosus* and 0·6 % phenol, 434 bacteria per unit volume would be reduced to 28 in about 4·25 minutes.

In these three experiments the concentration of bacteria was almost identical. If however this had not been so, the actual time necessary for a reduction in numbers of *B. paratyphosus* similar to that of the anthrax spores could have been obtained from the drawn curves showing rate of decrease of numbers of *B. paratyphosus* during disinfection with 0·6 % phenol (see figs. 7 and 9, H. C. 1908, p. 105) or from the formula expressing the same relation (p. 95).

The two series of experiments are still not directly comparable, for the time of disinfection of the anthrax spores was 25·5 hours and that of the similar reduction in number of *B. paratyphosus* was 4·25 minutes. It is necessary to find out what concentration of phenol would perform the disinfection of the same number of *B. paratyphosus* in 25·5 hours.

The logarithmic relation existing between the concentration of a disinfectant and the time taken for disinfection has recently been suitably expressed by H. E. Watson (1908) by the equation.

$$K \log C + \log t = \text{constant},$$

where K is some constant depending on the nature of the disinfectant and in some cases also of the organism employed. For carbolic acid and *B. paratyphosus*,

$$K = 5{\cdot}5,$$

and we have the equation

$$5{\cdot}5 \log C + \log t = \text{constant}.$$

Therefore $5{\cdot}5 \log 0{\cdot}6 + \log 4{\cdot}25 = 5{\cdot}5 \log x + \log 1530$

from which $x = 0{\cdot}206$,

and the concentration is 0·206 °/₀. The ratio of the concentration required to kill anthrax spores to that required in the case of *B. paratyphosus* is as 5 to 0·2, that is 25 times as strong.

Staphylococcus pyogenes aureus and anthrax spores. Krönig and Paul (1897) and Paul and Prall (1907) have made enumeration experiments with phenol upon anthrax spores and *Staphylococcus pyogenes aureus* respectively, and among these we have found two sets of observations where the reduction in numbers of the two species happens to be almost identical.

It is, strictly speaking, not allowable to institute a comparison between two such experiments, as no data are to be obtained as to the concentration of the bacteria during disinfection. Both sets of authors employed the garnet method (Krönig and Paul, 1897, pp. 7—11), in which the organisms are submitted to the action of the disinfectant in a thin film dried upon the surface of the garnets. For any series of experiments, in which the same emulsion of organisms is used for preparing the garnets in the first instance, it may be concluded that the concentration of bacteria during disinfection will be constant[1] also. It is less permissible to compare experiments made with different organisms, and at different times, even though the details of the method remain the same. At the same time, the probability is that, since in both cases the concentration of bacteria approached a maximum, we shall not obtain very erroneous results by comparing the two experiments.

In the one case (Krönig and Paul, 1897, p. 93) about 1000 anthrax spores were reduced to 260 in 25 hours by 5 °/₀ phenol at 18° C.; in the other (Paul and Prall, 1907, p. 116) 1040 staphylococci were reduced to 205 in 2½ minutes by 0·94 °/₀ phenol at 18° C.

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[1] That this is the case is shown by the fact that the laws shown to govern the disinfection process have been as accurately deduced from experiments using the garnet method (Krönig and Paul (1897), Madsen and Nyman (1907)) as from those in which the constancy of the concentration of bacteria was assured (H. C. 1908).

By calculation from the equation $5{\cdot}5^1 \log C + \log t = \text{constant}$, it appears that 0·29 % carbolic would reduce 1040 staphylococci to 205 in 25 hours, so that the relative concentration is approximately as 17 to 1. This figure is in fair agreement with the calculated result in the case of paratyphoid, for, as previously shown (Table VI), staphylococci possess a somewhat greater resistance towards phenol.

Mercuric chloride. It has not been possible to obtain any perfectly comparable data as to the comparative resistance of spores and vegetative forms towards mercuric chloride. However, with the reservation mentioned above, we propose to use again two sets of experiments with the garnet method in which anthrax spores and *Staphylococcus pyogenes aureus* respectively were disinfected.

TABLE VII.

	Test organism	Concentration of disinfectant B necessary to kill in 15 minutes, parts per 1000
Exp. 28. 5. 08	*Staphylococcus pyogenes aureus* A, old laboratory culture	2·0
,, 22. 5. 08	*Staphylococcus pyogenes aureus* B, virulent culture	2·4
,, 4. 6. 08	*B. typhosus* A, old laboratory culture ...	0·55
,, 4. 6. 08	*B. typhosus* B, which had then recently been passaged	0·80
,, 15. 7. 08	*B. typhosus* A	0·59
,, 15. 7. 08	*B. typhosus* B after 41 days' culture upon artificial media	0·56

In the former case 82 anthrax spores were reduced to 19 in 2 minutes by 1·69 % mercuric chloride at 18° C. (Krönig and Paul, 1897, p. 24). In the latter case, from three experiments (Paul and Prall, 1907, pp. 103, 104, and 105) 98 staphylococci were reduced to about 22 by 0·053 % mercuric chloride, also in 2 minutes at 18° C.

In this instance temperature of disinfection, the number (and presumably the concentration) of bacteria disinfected and the time taken all being the same, comparison of the concentration of disinfectant in the two cases gives direct indication of the relative resistance to mercuric chloride of anthrax spores and *Staphylococcus pyogenes aureus*; this ratio is as 32 to 1[2].

[1] The constant K happens to be the same in the case of phenol for both *B. paratyphosus* and *Staphylococcus pyogenes aureus*.

[2] In the case of mercuric chloride, it has been shown that mercuric ions are the real disinfecting agent (Krönig and Paul, 1897; H. Chick, 1908; Watson, 1908). Figures

Differences in resistance to disinfectants between virulent and avirulent strains of the same organism.

After cultivation upon artificial media a certain loss of resistance towards disinfectants was noticed in the case of *B. typhosus* and *Staphylococcus pyogenes aureus,* when compared with freshly isolated virulent strains. The experimental results are set forth in Table VII above.

Summary of Chapter V.

1. In the case of vegetative organisms a disinfectant varies in efficiency as much as ten times according to the organism against which it is tested. Some disinfectants are more efficient against one vegetative species of bacteria, others against another.
2. In the case of spores, metallic salts are the most efficient germicides. The action of phenol and emulsified disinfectants is too feeble for practical use.
3. The ratio between the concentration of phenol required to kill the same number per unit volume of sporing and vegetative forms in the same time varied according to the particular organism employed between 17 and 25 to 1.
4. A virulent strain of any particular species is generally somewhat more difficult to kill than a non-virulent strain.
5. Owing to the want of constancy as regards disinfection shown by various species, it is necessary to fix upon one particular organism for use in testing the germicidal value of disinfectants.
6. *B. typhosus* occupies an intermediate position in many respects, and has come to be employed in this country for the standardisation of disinfectants. It possesses the advantage of forming a fairly uniform suspension in broth culture and it is an organism which it is frequently desired to destroy in practice.
7. There is no particular reason why a virulent strain of *B. typhosus* should be employed in standardisation, and its use for the purpose would entail an unnecessary amount of danger.

CHAPTER VI. INFLUENCE OF ORGANIC MATTER.

(1) *Introductory.*

The practical value of the results obtained by any method of standardising disinfectants may justly be questioned if the comparison is

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expressing the relative concentration of Hg^{++} ions, corresponding to concentrations of the salt in the above experiments, were obtained from the results of Kahlenberg (1901) and Luther (1904); the ratio of the concentrations of Hg^{++} ions in the two cases is as 1·5 to 1.

made in the absence of organic matter, since in practice disinfectants are commonly used in its presence. The importance of this is evident when it is realised that, although presence of organic matter lessens the efficiency of every chemical disinfectant with which we have worked, the interference is to a different degree in every special case, depending (1) on the chemical nature and physical condition of the disinfectant, and (2) on the nature and condition of the organic matter.

Kenwood and Hewlett (1906) showed that in the presence of urine and faeces the value of phenol (Rideal-Walker method) was barely impaired, while emulsified disinfectants were reduced to two-thirds and one-half of their original value. These authors therefore suggest that the Rideal-Walker coefficient may in certain cases give a misleading value and that in any useful method of standardisation organic matter should be introduced. The sum of opinion, as the result of a long controversy[1] lasting over the last few years, is that it is desirable to introduce organic matter into any standard method of comparing the germicidal value of disinfectants, but great difficulty has been experienced in selecting the most suitable material.

It is impossible to arbitrarily select some convenient organic material without doing injustice to one or other class of disinfectant, so that one has to fall back upon some form which the disinfectant may encounter in practice. The use of faeces has the advantage of an approximation to the conditions of disinfection in many cases; it has, however, been objected to by Somerville and Walker (1906, I and II) as being too irregular in composition and therefore too uncertain in its effect upon disinfection to be adopted. Somerville and Walker suggest in a later paper (1907) the introduction of some standard organic matter, such as protein or gelatin, *in solution.*

In the case of many disinfectants, however, the physical condition of the organic matter present is an important factor in its effect upon the disinfectant, so that any method of standardisation which introduced organic matter only in solution would be inadequate, as in practice contaminating organic matter is partly in a particulate form. Wynter Blyth (1906) considers the question of organic matter both in solution and suspension, and notes the especial effect of the latter (e.g. faeces) in reducing the efficiency of emulsified disinfectants. He considers faeces, however, to be too inconstant in composition to be included in any standard test, but finds that a similar effect is produced by the addition of milk and recommends that milk should be adopted as a standard organic matter on the score of its convenience and approximate constancy in composition.

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[1] Full abstract is to be found in *Public Health Engineer*, May—Aug. 1906, and in the *Local Government Officer*, Sept. 1906 to May 1907.

We have made experiments to determine the effect of the presence of various kinds of organic matter both in solution and in suspension upon the germicidal value of a variety of disinfectants.

These experiments were undertaken with the view to ascertain how far the efficiency of disinfectants was modified by the conditions obtaining when they are added as a second line of defence to sera, vaccines, etc.; or when employed to disinfect dejecta.

Two methods were employed for estimating the effect of organic matter upon the germicidal value of disinfectants.

Method 1. In these experiments the time of disinfection was constant. Two concentrations (C_1 and C_2) which disinfected in 15 minutes, in distilled water and in presence of organic matter respectively, were directly determined, and the relative efficiency of the disinfectant in the two cases expressed as the ratio $\frac{C_2}{C_1}$.

Method 2. In the case of mercuric chloride we had not sufficient data available for an adequate investigation of the effect of serum in different concentrations and a second method was adopted which was less direct and involved rather more calculation.

The concentration (C_1) of a disinfectant was kept constant and the time (t_1) of disinfection of a certain number of organisms in unit volume was measured, when no organic matter was present, and the time (t_2) was also measured, when organic matter was present. It is possible to estimate the concentration C_2 of disinfectant, which would kill in the time t_2, if no organic matter had been added, by means of the curves showing the relation between concentration and time taken (H. C., 1908), or the result can be calculated by means of the formula $K \log C + \log t = K$, which has been shown by Watson to express these curves. The ratio $\frac{C_2}{C_1}$ expresses the efficiency of the disinfectant in presence of the organic matter if the efficiency in distilled water is reckoned as unity.

The following is an example of the calculation of relative efficiency in a case where the addition of organic matter was in the form of 10 % blood serum. In an experiment with phenol and *B. paratyphosus* it was found that 10 per 1000 phenol disinfected 30 millions of *B. paratyphosus* in 5 c.c. in 3 minutes. When 10 % serum was present the time taken was 12·5 minutes. Watson (1908) has shown that in the experiments of one of us with phenol and *B. paratyphosus* $5{\cdot}5 \log C + \log t = \text{constant}$.

Therefore $5{\cdot}5 \log 10 + \log 3 = 5{\cdot}5 \log C_2 + \log 12{\cdot}5$, where C_2 = concentration of phenol which would disinfect in 12·5 minutes if no serum had been present. Solving this equation we obtain $C_2 = 7{\cdot}7$, and the relative efficiency of phenol with and without 10 $^0/_0$ blood serum, $\frac{C_2}{C_1} = 0{\cdot}77$.

(2) *Organic matter in solution.*

Blood serum was chosen as a type of soluble organic matter which would be present in certain cases of disinfection. Its influence upon the germicidal value of disinfectants when present in concentrated form is also important, because disinfectants are frequently added to antitoxic sera as an additional precaution against contamination.

Experiments with 10 $^0/_0$ *blood serum.* The effect of 10 $^0/_0$ blood serum upon the efficiency of disinfectants was investigated by the two methods already described.

The effect of serum was determined in the case of phenol, mercuric chloride and the emulsified disinfectant "B," using *B. paratyphosus* and *B. typhosus* as test organisms. The results obtained are set forth in the tables below.

TABLE VIII.

Showing relative efficiency of various disinfectants with and without the addition of 10 $^0/_0$ *blood serum.*

Experiments made with Method 1.

Date of exp.	Test organisms	Disinfectant	Concentration required to kill in 15 minutes, parts per 1000		Relative efficiency, (concentration required in distilled water = 1)
			In distilled water	In presence of 10 $^0/_0$ serum	
12. 11. 07	*B. paratyphosus*	Phenol	8	9	0·89
14. 11. 07	,,	,,	7·75	8·25	0·94
26. 5. 08	,,	,,	8·75	10·5	0·83
21. 5. 08	*B. typhosus*	,,	7·75	9·5	0·87
13. 11. 07	*B. paratyphosus*	Disinfectant "B"	0·7	1·0	0·70
15. 11. 07	,,	,,	0·625	0·85	0·77
15. 11. 07	,,	Mercuric chloride *	about 0·05	about 0·45	about 0·11
	,,	,,	,, 0·10	,, 0·40	,, 0·25

* For reasons explained above, p. 673, the error in the determination of the germicidal value of mercuric chloride is 100 $^0/_0$.

TABLE IX.

Showing the relative efficiency of mercuric chloride when employed as disinfectant in the presence of various concentrations of blood serum.

Experiments made with Method 2.

Organism	Initial concentration of $HgCl_2$ parts per 1000	Nos. expressing initial concentration of Hg^{++} ions	Concentration of blood serum %	Time taken for disinfection, minutes	Nos. expressing effective concentration of Hg^{++} ions	Relative efficiency in terms of concentration of Hg^{++} ions	Effective concentration of $HgCl_2$, parts per 1000	Relative efficiency in terms of concentration of $HgCl_2$
Exp. 9. 11. 07.								
B. para-	0·5	57·5	—	7·2	57·5	1·0	0·5	—
typhosus	0·5	57·5	5	10	52·7	0·91	0·30	0·60
	0·5	57·5	10	14·2	48·1	0·84	0·18	0·36
	0·5	57·5	20	39	36·9	0·64	0·05	0·10
	0·5	57·5	30	62	32·6	0·56	0·03	0·06

In the case of phenol the efficiency of the disinfectant is reduced about 12 % in the presence of 10 % blood serum, and with the emulsified disinfectant "B," the reduction is rather greater. The results with corrosive sublimate (see Table IX) require a few words of explanation. In calculating the concentration of $HgCl_2$, which in the absence of serum would disinfect in the time observed, it is necessary to express the original concentration of sublimate in terms of concentration of Hg^{++} ions. This was done by means of a curve constructed from the figures of Luther and Kahlenberg already referred to (p. 677 footnote). The calculated concentration, which in the absence of the serum would operate in the same time, was therefore also in terms of concentration of Hg^{++} ions and had to be again translated back into concentration of $HgCl_2$ by the same means. The effect of adding blood serum to a solution $HgCl_2$ is to precipitate some of the mercuric salt as an albuminate which thereby lowers the concentration of mercuric chloride, but, owing to the peculiar ionisation of this salt, the concentration of Hg^{++} ions varies but slowly with alteration in concentration of the salt.

Effect of blood serum in high concentration. A few experiments were made with *B. paratyphosus*, but this organism was abandoned because it was found to be particularly susceptible to the bactericidal action of horse serum. This was still very marked after the serum had been heated to 60° C. for one hour.

The effect of serum in high concentration upon the germicidal value of phenol was therefore studied with *Staphylococcus pyogenes aureus*.

In spite of the fact that the presence of the serum lessens the efficiency of the disinfectant, it was found that 0·25 % carbolic acid disinfected a liberal seeding (6,000,000 per c.c.) of staphylococcus in the presence of serum (previously heated to 60° C.) in less than two weeks at laboratory temperature (Table X). The disinfection was rendered much more rapid at 37° C., and in this case the staphylococci added were killed in two or three days.

TABLE X.

Showing the germicidal effect of dilute solutions of phenol upon staphylococcus pyogenes aureus in the presence of 90—95 % *blood serum previously heated to* 60—70° C.

Experiments made with Method 1.

Date of exp.	Temperature, degrees centigrade	Concentration of disinfectant parts per 1000	Concentration of blood serum %	Time taken for disinfection in presence of serum	Time taken for disinfection in distilled water
18. 11. 07	20	5	90	2 days	3 hours
	20	2·5	95	9 days	3 days
27. 11. 07	20	5	90	5·5 days	2 hours
	20	2·5	95	more than 12 days	23 hours
27. 3. 08	20	2·5	95	11 days	8 days
	37	2·5	95	30 hours	greater than 8, less than 24 hours
30. 3. 08	20	2·5	95	10·5 days	8 days
	37	2·5	95	50 hours	30 hours

(3) *Experiments with particulate organic matter of various kinds.*

a. Animal charcoal. As might be expected the influence of animal charcoal varies very considerably both according to the nature of the germicide and its condition, viz. whether in solution, e.g. phenol, or in suspension, as in the case of emulsified disinfectants.

50 c.c. of 1 % phenol was shaken up with 1 grm. of charcoal and the filtrate was found to contain 0·74 %[1]. The same amount of charcoal reduced the concentration of 50 c.c. of 5 % phenol to 3·85 %, the amount removed by the charcoal being nearly proportional to the concentration of the original solution. This suggests that the process is one of adsorption[2].

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[1] Estimated with bromine.

[2] The first portion of adsorption curves are not uncommonly nearly linear, so that observations confined to a small range of low concentrations show an approximation to proportionality.

With emulsified disinfectants, the proportion removed is much greater. 0·6—0·8 grm. charcoal completely removed the tar-acids from 50 c.c. of an emulsion of disinfectant "A," containing 5 parts in 1000 (2·5 parts per 1000 of tar-acids) so that the filtrate was perfectly clear. In the case of disinfectant "B" 1 grm. charcoal added to 25 c.c. of 5 in 1000 disinfectant (3 per 1000 of tar-acids) gave a filtrate with only a trace of opalescence. In these observations the germicidal value of the filtrates was also directly determined, and it was found that in the case of "B" loss of germicidal power accompanied loss of opacity. Disinfectant "A" contained kresols in addition to higher tar-acids, and in this instance the clear filtrate still possessed one-tenth of its original potency.

The above facts suggest that the removal of an emulsion of tar-acids by animal charcoal might be a case of adsorption. This action of animal charcoal was therefore made the subject of special study and the quantitative relationships between the amount removed and the original concentration determined. The proportion of animal charcoal used was comparatively small, so that a considerable proportion of emulsified tar-acids were left unadsorbed at the end of the experiment. The residual tar-acids after treatment with animal charcoal were estimated. The following method was tested and found to yield sufficiently accurate results.

0·5 grm. animal charcoal (Kahlbaum's purest) was added to 20 c.c. of a series of concentrations of emulsified tar-acids varying from 0·23 to 1·39 %. The mixtures were allowed to remain in contact 3 hours in a shaking apparatus. At the end of this period they were filtered and a small proportion (3 to 10 c.c.) of the filtered solution was taken for analysis in each case. The sample was placed in a small separating funnel, acidified with hydrochloric acid, and shaken with ether, in which tar-acids are readily soluble. The watery layer was drawn off and the ether extract was dried by the addition of calcium chloride and withdrawn into a weighed beaker. The calcium chloride was washed with dry ether and the washings added. The ether was evaporated, first on a water-bath and finally on a warm plate at a temperature of 60°—70° C., and the residue weighed.

The results of these experiments, given in Table XI, show that the amount of emulsified tar-acids removed by animal charcoal is not constant. It is at first nearly proportional to the original concentration of tar-acids, but, as this concentration increases, its influence becomes progressively less and the amount adsorbed rapidly reaches a maximum. The maximum amount that the animal charcoal took up was 20 % of its own weight.

TABLE XI.

Adsorption of emulsified tar-acids of different concentrations by animal charcoal.

Date of exp.	Concentration of animal charcoal %	Initial concentration of tar-acids %	Final concentration of tar-acids %	Quantity adsorbed by 1 grm. animal charcoal grms.
8. 10. 08	2·5	1·39	0·88	0·202
,,	2·5	1·16	0·75	0·200
,,	2·5	0·70	0·23	0·185
7. 10. 08	2·5	0·64	0·21	0·169
8. 10. 08	2·5	0·46	0·11	0·144
,,	2·5	0·35	0·043	0·122
,,	2·5	0·23	0·031	0·080

The amounts adsorbed by 1 gram of charcoal plotted against either the initial or final concentration of the emulsion give points which lie upon a regular curve of the same essential form as has been obtained in the case of other adsorption phenomena[1] (cf. Schmidt (1894); Bayliss (1906)).

b. Dust. The dust was obtained from the tops of cupboards and contained 39 % organic matter and 61 % inorganic residue. The effect of dust in reducing efficiency was tested upon an emulsified disinfectant only.

The results of experiments using method 2 (p. 679), are given in Table XII and it will be seen that with 3 % dust and concentrations of emulsified disinfectant "B" ranging from 1·5 to 4 parts per 1000, there is a reduction of efficiency to about one half, independent of the original concentration of the disinfectant.

With a bacteriological method the observations were necessarily limited to a small range of low concentrations, when, as shown by observations with animal charcoal, the amount of adsorption is approximately proportional to the concentration. This indicates that the action is of a physical and not of a chemical nature.

In an experiment made with *B. typhosus* and the disinfectant "B" at 20° C., using method 1, it was found that when 3 % dust was present a concentration of 1·4 per 1000 was required to disinfect in 15 minutes, whereas in distilled water 0·47 per 1000 was adequate, the effective concentration being reduced to 0·34 of the original. At the same time an experiment was made using 3 % dust which had previously been ignited to free it of all organic matter, and in this case 0·45 parts per 1000 of disinfectant "B" were required for 15 minutes' disinfection, the strength of the disinfectant not being impaired by the addition of the ignited dust. This experiment shows that it is the organic part of dust which alone is responsible for lessening the efficiency of emulsified disinfectants.

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[1] See this *Journal*, p. 702.

TABLE XII.

Effect of presence of dust upon emulsified Disinfectant "B."

B. paratyphosus 20° C. Disinfectant "B."

	Concentration of dust %	Concentration of disinfectant "B," parts per 1000	Time taken for disinfection	Calculated* effective concentration of disinfectant "B," parts per 1000	Relative efficiency (original concentration = 1)
Exp. 31. 10. 07	3	4	0·75 min.	2	0·50
	,,	3	2·75 mins.	1·13	0·38
	,,	2	6 ,,	0·92	0·46
	,,	1·5	27 ,,	0·75	0·50
	,,	1·0	76·5 ,,	—	—

* Experiments were made determining time of disinfection for a series of concentrations in distilled water; from the results of these experiments curves were drawn, and the concentrations given in column 4, corresponding to the times in column 3, were determined from the curves.

c. Coagulated horse serum. Coagulated horse serum was used in the form of a very fine precipitate. The serum was coagulated by heat, after dilution with distilled water in such a proportion that the concentration of the suspension was 0·66 %. 15 c.c. of this suspension was mixed with 5 c.c. of disinfectant "B" (2 per 1000). The resulting fluid yielded therefore a suspension of 0·1 grm. proteid in 20 c.c. of an emulsion of disinfectant "B" in the strength of 0·5 in 1000. After centrifuging, the mixture was found to be completely freed from all opacity by the addition of this small quantity of coagulated proteid.

In another experiment 20 c.c. of 0·5 in 1000 disinfectant "B" was completely cleared by the presence of ·07 grm. of the same coagulated serum.

The disinfectant "B" contained 39·2 % of water so that in the above cases the finely divided coagulated albumen had appropriated at least 8 % of its own weight of tar-acids, and this under circumstances which from analogy with the results with animal charcoal (Table XI) must be regarded as the least favourable.

d. Bacteria, dead and alive. A similar phenomenon was observed when suspensions of bacteria, dead or alive, were mixed with solutions of emulsified disinfectants. While a control tube containing the emulsified disinfectant showed little or no settlement on centrifuging, the tube containing bacteria was cleared of all opacity and disinfectant properties if the relative quantities of disinfectant and bacteria were suitably arranged. For example, when 1 c.c. of an emulsion of *B. paratyphosus* was mixed with 1 c.c. of a solution of disinfectant "B," containing 0·5 part per 1000, the resulting fluid after centrifuging was clear.

In experiments, in which the quantitative relations were studied, the same general results were obtained as with animal charcoal, viz. the amount of tar-acids removed by the bacteria was at first proportional to the concentration and then rapidly reached a maximum. It was found that bacteria could under favourable circumstances remove 39 °/₀ of their own dry weight of tar-acids from an emulsion.

These facts are capable of explaining the high efficiency of this class of disinfectant and are dealt with in detail in a separate communication[1].

(4) *Influence of the presence of a suspension of faeces upon the various types of disinfectants.*

The bulk of the disinfectants manufactured are destined for the disinfection of excreta, drains, etc. where they have to operate in the presence of more or less faecal matter. It is therefore of interest to learn to what extent the action of various classes of disinfectants may be affected by the presence of faeces.

The fact that the germicidal value of emulsified disinfectants is seriously reduced in the presence of faeces has been pointed out by Kenwood and Hewlett (*loc. cit.*) and Wynter Blyth (*loc. cit.*). Fowler (1906) also made some experiments with a 5 °/₀ extract of fresh faeces in equal parts of urine and water. The effect upon phenol and emulsified disinfectants of this very dilute watery extract of faeces was found to be negligible. As however all particulate matter was filtered off through paper, Fowler's results do not really bear upon the matter at present under discussion.

The following table (Table XIII) shows the effect upon the efficiency of different classes of disinfectants of the addition of faeces. The concentration of various disinfectants, which killed 6,000,000 per c.c. *B. typhosus* in 15 minutes at 20° C., was determined (*a*) in distilled

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[1] This *Journal*, p. 698.

TABLE XIII.

Disinfectant	Active principle	Concentration required to disinfect about 6,000,000 per c.c. *B. typhosus* in 15 minutes at 20° C. (a) In distilled water, parts per 1000	(b) In presence of 3 % dried faeces, parts per 1000	Relative efficiency in presence of 3 % faeces as reckoned by ratio $\frac{\text{concentration } a}{\text{concentration } b}$	Remarks
(1) $HgCl_2$ ...	...	5	50	0·10	—
(2) Commercial disinfectant	$HgCl_2$ ...	0·13	1·5	0·09	—
(3) Commercial disinfectant "Cresol" No. 3	Cresols and higher tar-acids	0·55	6·0	0·09	Contains resin soap as emulsifier. Fine emulsion on dilution with water.
(4) Commercial disinfectant "B"	Higher tar-acids mostly boiling between 210—260° C.	0·75	4·5	0·17	Contains resin and soft soap as emulsifier. Fine emulsion on dilution with water.
(5) Commercial disinfectant "Cresol" No. 2	Cresols and higher tar-acids	2·5	8·5	0·29	Contains resin soap as emulsifier. Coarse emulsion on dilution with water.
(6) Commercial disinfectant "Cresol" No. 1	Cresols and higher tar-acids	5·0	15·0	0·33	Contains resin soap as emulsifier. Coarse emulsion on dilution with water.
(7) Commercial carbolic acid	91 % Cresols ...	2·2	4·2	0·52	1 grm. almost completely soluble in 100 c.c. water.
(8) Commercial carbolic acid	95 % Cresols ...	3·2	4·8	0·66	1 grm. almost completely soluble in 100 c.c. water.
(9) Phenol ...	...	8·0	9·25	0·86	—

water, (*b*) in water containing 3 per cent. by weight of a sample of dried sterile faeces.

The faeces were obtained from an individual upon an ordinary mixed diet. They were dried at 105° C., powdered and passed through a fine sieve and the appropriate quantity weighed out and added to the tubes.

The fact that the presence of organic matter with which it can combine should seriously reduce the disinfecting power of mercuric chloride requires no comment. Phenol, a completely soluble disinfectant, is reduced in value about 10 % by the presence of 3 % faeces. The large effect upon disinfectants, in which the active principles (cresols and tar-acids) are in the form of an emulsion, demands some explanation. The germicidal value of tar-acids is, other things being equal, dependent in some way upon the excellence of the emulsion, and, from the results in Table XIII above, it appears that the amount of interference by faeces is also related to the fineness of the emulsion; in other words, *the higher the quality of the disinfectant in distilled water due to fineness of emulsion, the more it is interfered with in the presence of particulate organic matter.*

The clue to an interpretation of the deterioration of these emulsified disinfectants in the presence of faeces is afforded by the similar effect of charcoal, dust, coagulated albumen and bacterial suspensions upon this class of disinfectant.

From these observations it appears to be the particulate nature of the organic matter which is responsible for this deleterious influence upon emulsified disinfectants, and the quantitative experiments indicate that the process which takes place is one of adsorption of the fine particles of the emulsion.

Experiments were made to ascertain what quantitative relation existed between the reduction in germicidal value and the original concentration of the emulsion, when the amount of faeces was kept constant. The results of these experiments are set forth in Table XIV and show that the same percentage reduction is produced with concentrations of disinfectant varying between 3 and 7 per 1000.

The results with faeces thus fall into line with those obtained with charcoal, dust and bacteria for similar strengths of emulsion and can also be explained as due to adsorption. Since, however, the emulsified tar-acids in disinfectants "A" and "B" are soluble in olive oil and the sample of faeces used contained 24·3 % ether extract, it was conceivable that some portion of the action of faeces might be due to removal of the emulsion by its solution in the fat of the faeces. If this occurred, proportionality between amount removed and the concentration of disinfectant would also be maintained. Accordingly, experiments were made with faeces from which the fat had been extracted and it was found that the action of the extracted faeces was, weight for weight, only slightly less marked than that of the normal faeces (Table XIV). This action of faeces is therefore only due in small part to the solvent action of the contained fat.

It was possible that the loss might be partly or wholly due to a de-emulsifying action of the soluble organic matter present in faeces. Accordingly the dark coloured fluid obtained from a 3 or 6 % suspension of faeces in distilled water was tested for any de-emulsifying action upon the disinfectant "B." This fluid was mixed with a dilution of "B," but no settlement was detected either upon standing or after centrifuging.

TABLE XIV.

Quantitative relationship between original concentration of emulsion and depreciation in germicidal value due to presence of faeces.

Date of exp.	Concentration and nature of faeces	Initial concentration of emulsion, parts per 1000	Time taken for disinfection, 5 drops of 24 hrs. culture of *B. paratyphosus* in 5 c.c., minutes	Calculated effective concentration, parts per 1000	Relative efficiency, initial concentration =1
(1) 26. 10. 07	3 % dried faeces	6	2·5	1·2	0·20
		5	3	1·15	0·23
		4	13	0·87	0·22
(2) 26. 10. 07	3 % dried fat extracted faeces	5	1·5	1·4	0·28
		4	2·5	1·2	0·30
		3	13	0·87	0·29
(3) 26. 10. 07	2 % dried faeces	7	—	1·00	0·14
		6	—	0·9	0·15
		5	—	0·74	0·15
		4·1	—	about 0·55	0·13

Experiments 1 and 2 were made with Disinfectant "B," Experiment 3 with Disinfectant "A."

The action of faeces in reducing the strength of emulsified disinfectants must therefore be attributed principally to a surface action between the particles of the emulsion on the one hand and those of the faeces on the other, resembling closely the action between the other three types of particulate matter investigated, dust, animal charcoal and coagulated blood serum.

Summary of Chapter VI.

1. The presence of 10 % blood serum reduces the efficiency of 1 % phenol about 12 %. The effect upon emulsified disinfectants is somewhat greater. With mercuric chloride the reduction was much greater, a 0·5 % solution being reduced to from 0·6 to 0·06 of its original value as the concentration of serum was increased from 5 to 30 %.

2. Experiments made with more concentrated serum showed that thirty million staphylococci added to 5 c.c. of the serum containing 0·25 % phenol were killed in less than two weeks at a temperature of 20° C. and in 30 to 50 hours at a temperature of 37° C.

3. The presence of particulate organic matter—animal charcoal, dust, finely precipitated coagulated albumen, bacteria, and faeces—affect

the germicidal value of emulsified disinfectants containing tar-acids to a much greater extent than that of solutions of phenol.

4. The whole of the emulsified tar-acids can be removed by a suitable addition of any of the above forms of particulate organic matter.

5. The amount of tar-acids removed by animal charcoal from an emulsified disinfectant is at first proportional to the original concentration of tar-acids, but, as this concentration increases, the proportion removed rapidly diminishes. The amounts removed by a given quantity of charcoal from different strengths of emulsion increase at first rapidly and then more and more gradually until, with an emulsion of 1·4 % tar-acids, the maximum is nearly reached. The curve drawn from the observations, tabulated in Table XI, presents the usual form of an adsorption curve (see this *Journal*, p. 702, Fig. 1).

6. The removal of an emulsion of tar-acids by bacteria obeys the same quantitative laws as obtain in the case of animal charcoal.

7. The removal of tar-acids by dust was only investigated over a limited range of initial concentration and was found to take place in accordance with the same general law.

8. The effect of a 3 % suspension of finely powdered dried faeces upon the efficiency of phenol was to reduce it about 10 %; upon commercial cresols a reduction of 30 to 50 % was produced, depending upon the completeness with which the preparation dissolved. The freer the sample was from higher and insoluble homologues, the less depreciation in the original value occurred.

9. The effect upon various emulsified disinfectants containing higher tar-acids was to reduce their efficiency to a value varying from $\frac{1}{3}$rd to $\frac{1}{11}$th of the original. The finer emulsions were more seriously reduced in value than the coarser ones.

10. Within the limits of concentration of disinfectant employed, the amount of emulsion removed from the liquid by powdered faeces was nearly proportional to the concentration of disinfectant. In this case, as with the other forms of particulate organic matter, the removal was shown to be principally due to adsorption of the emulsion upon the surfaces of the fine particles.

Chapter VII. A method of standardising disinfectants in the presence of faeces.

It might be imagined that faeces would prove an impossible material to be included in a standard test on account of inconstancy in composition. This has been contended by Wynter Blyth (*loc. cit.*) and Somerville and Walker (*loc. cit.*), and is certainly true if the faeces are employed in their natural condition, but has not been found by us to be the case if they are dried and powdered. Our experience on the contrary has shown that, when dried and ground to a fine powder, faeces from different individuals upon an ordinary mixed diet display a surprising uniformity with regard to the extent to which they influence the germicidal power of a disinfectant.

The particulate matter in human faeces consists largely of bacteria and portions of undigested food, together with varying quantities of fat and other bodies soluble in ether. In addition, a considerable amount of the pigment hydrobilirubin is present in an insoluble form, combined with calcium. These together account for about 70 °/₀ of the total dry weight. In the preceding chapter we have shown that the reduction of the efficiency of emulsified disinfectants by faeces is caused by the solid particles contained therein and is principally due to adsorption, hence depending as much upon the physical condition of the faeces as upon their chemical composition.

The following experiments (see Tables XV and XVI) illustrate the effect of 15 different samples of faeces, dried at 105° C. and ground to a fine powder, upon the germicidal value of emulsified disinfectants. In the experiments with *B. typhosus* (Table XVI) the variation caused by the different samples of faeces is seen to be within the error of the method, for the divergence between the results obtained upon the same day with faeces from different individuals was not greater than those obtained with the same sample of faeces upon different days.

TABLE XV.

Emulsified disinfectant "B" with various samples of faeces.

Disinfection of about 6,000,000 *B. paratyphosus* per c.c. (5 drops from a 24 hours' culture at 37° C.).

Sample of faeces	Concentration of "B" required for complete disinfection in 15 mins. at 20° C. in presence of 3 °/₀ faeces, parts per 1000
I	5·5
II	5·5
III	5·0
IV	4·5
V	5·0

TABLE XVI.

Emulsified disinfectant "B" with various samples of faeces.

Disinfection of about 6,000,000 *B. typhosus* per c.c. (5 drops from a 24 hours' culture at 37° C. in 5 c.c.).

Concentration of "B" required for complete disinfection in 15 minutes at 20° C., in presence of 3 % faeces, parts per 1000.

Sample of faeces	Date of exp.:						
	21. 3. 08	28. 3. 08	29. 4. 08	5. 5. 08	7. 5. 08	8. 5. 08	9. 5. 08
II	—	—	—	—	—	4·0	—
V	—	—	—	—	—	—	4·5
VI	—	—	—	4·8	—	—	—
VII	—	—	4·4	—	—	4·0	—
VIII	—	—	—	—	—	4·6	—
C	5·2	4·8	—	4·3	4·0	more than 6	—
D	—	—	—	—	—	4·0	—
E	—	—	—	—	4·6	—	—
F	—	—	—	—	—	—	4·3
G	4·4	4·1	—	—	—	—	—
H	4·1	—	—	—	—	—	4·9
I	—	—	—	4·5	4·3	—	4·3

TABLE XVII.

Mercuric chloride with various samples of faeces.

	Samples of faeces	Concentration of mercuric chloride required for disinfection of about 6,000,000 per c.c. *B. typhosus* in presence of 3 % faeces at 20° C. in 15 minutes, parts per 1000	Nos. expressing concentration of mercuric ions
Exp. 23. 7. 08	VII	1·0	63
	VIII	1·0	63
	IX	1·5	68
	II	1·5	68
	I	2·0	71
	I	2·0	71

The same uniformity of effect was apparently not maintained when the experiments were made with mercuric chloride, but here again the discrepancy is really within the experimental error. As shown in Table XVII the concentration necessary to disinfect 6,000,000 per c.c. *B. typhosus* in 15 minutes varied between 1 and 2 parts per 1000. This is not however of material significance, because the error of determining the germicidal value of mercury in this concentration is about 100 %, owing to the fact that, although the concentration of mercuric chloride changes from 1 to 2, the concentration of mercuric ions only varies as 63 to 71.

Description of the method employed.

The essential features of the method which we have been in the habit of using are those of the Rideal-Walker process, but a constant time, 30 minutes, is allowed for the disinfectant to act. The introduction of the faeces somewhat increases the experimental error which if we exclude one aberrant result in Table XVI is now less than 20 %, whereas in distilled water (see p. 665) we could rely upon an error not exceeding 10 %.

The faeces used were dried first in a water bath and subsequently at 105° C., ground to a fine powder, and passed through a fine sieve with a mesh of 130 to the inch. Quantities of 0·15 grm. were weighed out and placed in test tubes. To each test tube 2·5 c.c. distilled water was added and the tube sterilised in the autoclave (10 minutes at 120° C.). The tubes were covered with indiarubber caps and kept in jars with greased lids to prevent evaporation.

At the time of the experiment different amounts of a suitable dilution of the disinfectant were added to each tube together with enough distilled water to make the total volume up to 5 c.c. The tubes then contained different concentrations of the disinfectant in question in the presence of 3 % faeces[1]. The tubes were inoculated and sampled in exactly the same way as when the test was made in distilled water (see p. 665).

In the case of an unknown disinfectant one or two preliminary trials are necessary to ascertain a suitable range of concentrations to give a useful result. The exact procedure may be made clearer by an actual example.

Six tubes each containing 0·15 gr. faeces in 2·5 c.c. of distilled water having been prepared, to each is added varying quantities of 2 % disinfectant and water as in Table XVIII below, so arranged that the tubes contain 0·5 c.c. of a series of concentrations of disinfectant in the presence of 3 % faeces. In this experiment the approximate concentration necessary to disinfect in 15 minutes has been determined previously, and the actual concentration required to disinfect in the time is found to be between 4 and 4·6 parts per 1000.

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[1] Three per cent. faeces was chosen as representing the amount of solid matter present if a liquid stool (containing 10 % solids) were mixed with twice its volume of the disinfectant.

An exactly similar experiment with pure phenol is simultaneously made, so that the carbolic acid coefficient of the disinfectant can be directly determined.

TABLE XVIII.

Faeces I. 3 °/₀, and a constant number of *B. typhosus* per c.c.

	No. of c.c. of 2 °/₀ disinfectant	No. of c.c. distilled water added	Total volume added, c.c.	Concentration of disinfectant in the tube, parts per 1000	Result of experiment + = acid formation in test cultures* in glucose broth
Exp. 9. 5. 08	0·9	1·6	2·5	3·6	+ +
	1·0	1·5	2·5	4·0	+ +
	1·15	1·35	2·5	4·6	– –
	1·3	1·2	2·5	5·2	– –
	1·5	1·0	2·5	6·0	– –
	1·7	0·8	2·5	6·8	– –

* Test cultures were always made in duplicate.

This method in one way departs from the methods of practical disinfection. A disinfectant is in practice added to a mixture of organic matter and bacteria; in the method above the disinfectant is previously mixed with the organic matter and the bacteria are added subsequently. It was conceivable that the latter order of mixing might impose a harder test upon the disinfectant than was necessary. Comparative experiments were therefore made using either method, in the one case the test was carried out as already described, in the other case the bacteria were introduced into the solution of faeces and the disinfectant added to this mixture. No significant difference was found between experiments using the two methods.

	Disinfectant added before culture. Concentration necessary for 15 minutes disinfection, parts per 1000	Disinfectant added after culture. Concentration necessary for 15 minutes disinfection, parts per 1000
Exp. 16. 7. 07	5·0	4·4
Exp. 19. 7. 07	4·8	4·8

We are not particularly enamoured with the method of testing a disinfectant in the presence of three per cent. dried faeces. In many respects it departs from the ideal, but by its means the germicidal value of a disinfectant can be approximately measured under conditions such as it may not unlikely encounter in practice. We realise that disinfectants are usually employed in a very casual way and quite as commonly as deodorizers as with a view to the sterilisation of the germs of disease. But there are times when the medical man or sanitarian wants to know what reliance he can place upon this or that disinfectant for a particular purpose, and we think that a determination of the value of a disinfectant made in the presence of three per cent. faeces will, for the majority of purposes, be more valuable than when made in distilled water. Everyone, we presume, would agree as to the absurdity of forming an estimate of the utility of an oxidising disinfectant, such as permanganate of potash, by determining its germicidal value upon a few bacteria in distilled water, or to accept the statement that 1 in 10,000 parts of mercuric chloride would be a reliable disinfectant for pus, because this concentration will suffice in distilled water to destroy 6,000,000 *B. typhosus* per c.c. in 15 minutes at 20° C. Except on a few occasions, for surgical purposes, a disinfectant will have to act in the presence of more or less particulate organic matter, and we have chosen that form which will be present in one of the common cases for which disinfectants are employed.

Our observations have clearly shown that just as the value of oxidisers will, from their nature, be depreciated in the presence of oxidisable matter other than the bacteria, and mercurial preparations by the presence of proteid or other material with which mercury combines, so emulsified disinfectants from their very nature will be diminished in value by the adsorption of the emulsion of tar-acids upon a great variety of particles of organic matter, if such be present.

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A COMPARISON OF THE POWER OF A GERMICIDE EMULSIFIED OR DISSOLVED, WITH AN INTERPRETATION OF THE SUPERIORITY OF THE EMULSIFIED FORM.

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HENLE (1889) found that "Creolin," an emulsion of tar acids, was a more efficient disinfectant than could be explained from observations upon each of its constituents separately, and concluded that the extra efficiency was connected with the emulsified form. Henle's conclusions do not appear to us to be necessarily justified by his observations.

Rideal and Walker (1903, p. 425) state that an emulsion of Trikresol is equal in germicidal power to a solution three times as concentrated, but give no details.

We have compared the germicidal value of higher tar acids when emulsified in water and when dissolved in alcohol. The spores of *B. subtilis* were chosen, because alcohol alone was found to have no action upon them within the time limits employed.

A suspension of an old sporing culture of *B. subtilis* was made in water and heated for some minutes to 80° C. By a preliminary plating experiment an amount of spores suitable for enumeration experiments was determined, and this amount was added to two tubes containing 2·5 % tar acids in solution in alcohol and emulsified in water respectively. The tubes were placed in a water bath at 20° C. and a definite quantity removed from each at known intervals of time, and plated. After 24 hours' incubation the numbers of colonies on the plates were counted.

The numbers of colonies formed a regularly decreasing series in both cases, but diminished more rapidly in the case of disinfection by tar acids in the form of an emulsion (see Table I).

Disinfection has been shown by Madsen and Nyman (1907) and by one of us (H. C. 1908) to proceed according to the equation

$$\frac{1}{t_2 - t_1} \log \frac{n_1}{n_2} = K,$$

where t_1 and t_2 are any two times, and n_1 and n_2 the numbers of surviving organisms in unit volume of the liquid at times t_1 and t_2 respectively, and K a constant depending on the particular organism and disinfectant, the concentration of the latter, and the temperature.

This constant is therefore an expression for the velocity of the reaction.

From the number of bacteria surviving after various intervals of time (see Table I, col. 4) two values of K were calculated (see col. 5), the ratio of which expresses the relative velocity of the disinfection by the same concentration of tar acids in the two conditions.

The mean value for K was 0·049 for the emulsion and 0·0066 for the solution, so that coal tar acids in the above concentration disinfected

TABLE I.

Spores of B. subtilis. 20° C.

Disinfectant	Time elapsing, minutes	Amount of sample	Mean no. of bacteria present in 1 drop disinfecting mixture	K, assuming reaction to be in accordance with the equation $-\frac{dn}{dt}=Kn$ $\left(K=\frac{1}{t_2-t_1}\log\frac{n_1}{n_2}\right)$
Exp. 29. 2. 08				
2½ % tar acids emulsified in water	1	1 drop	2750	taken as an initial value of n $(=n_1)$ in calculating values of K.
	4·5	1 ,,	1804	0·052
	20	1 ,,	373	0·046
			Mean value of K	0·049
2½ % tar acids dissolved in alcohol	1	1 ,,	1750	taken as an initial value of n $(=n_1)$ in calculating values of K.
	70	1 ,,	607	0·0067
	121	1 ,,	393	0·0054
	230	1 ,,	32	0·0077
			Mean value of K	0·0066

7·5 times[1] as quickly in the form of an emulsion as when dissolved in alcohol.

The explanation of this increased efficiency of a germicide in the emulsified form demands some consideration. Fowler (1907) suggested that there is a mutual attraction between the particles of an emulsion and the bodies of bacteria. He was led to this conclusion from the examination of stained microscopical preparations containing mixtures of bacteria and emulsions; he then observed bacteria with particles of disinfectant adhering to them. On repeating Fowler's observations it was found that methylene blue, in addition to staining the particles, de-emulsified the disinfectant, so that conclusions drawn from appearances seen after its addition are not free from fallacy.

In order to arrive at an understanding of what takes place, we observed mixtures of living bacteria (*B. paratyphosus*) and an emulsified disinfectant, under a magnification of 1100 diameters, using a dark background and side illumination. Under these circumstances the bacteria and the particles of the disinfectant appear as shining bodies and their relation to one another can be easily observed.

The particles of the emulsion, and to a lesser degree the bacteria, exhibited active Brownian movement. The bacteria, which were considerably larger than the mean diameter of the emulsified particles, were seen to be continually bombarded by the latter. The bacteria were thus frequently brought into intimate contact with particles of tar acid somewhat smaller than themselves, but whether any portion was left remaining upon them could not be directly observed. Nothing of the nature of an agglutination of the particles upon the bacteria was seen, although an occasional temporary attachment was noticed.

Nevertheless, whereas the emulsified disinfectant alone showed a multitude of small shining bodies, an emulsion of equal concentration to which bacteria had been added showed a great reduction in the number of these bodies, so that many of the emulsified particles of the disinfectant had been appropriated by the bacteria.

This appropriation by bacteria of particles of tar acids from an emulsion was readily demonstrated by mixing together a suspension of bacteria and an emulsion of tar acids, allowing them to remain in contact a short time, and subsequently centrifuging. Under these circumstances the mixture was completely cleared of all opacity when the relative quantities of tar acids and bacteria were suitably arranged. A control tube containing the emulsion showed very little settlement on centrifuging.

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[1] This does not mean that the disinfectant in the emulsified form was, weight for weight, 7·5 times as efficient, see "An Investigation of the Laws of Disinfection," by H. Chick, this *Journal*, pp. 117—132.

The suspension of bacteria (*B. paratyphosus*) employed contained 0·025 grm. dry weight of bacteria per c.c. When 1 c.c. of this bacterial suspension was mixed with 1 c.c. of an emulsion containing 0·6 in 1000 of tar acids, so that the 2 c.c. of mixture possessed a concentration of 0·3 per 1000, a perfectly clear solution was obtained after centrifuging, i.e. 0·025 grm. bacteria united with 0·0006 grm. of tar acids, which is more than 2$^0/_0$ of its own weight.

In order to gain some further insight into the nature of the process a series of quantitative experiments were undertaken using a constant weight of bacteria and emulsions of varying concentration. *Staphylococcus pyogenes aureus* was employed. The cocci were three times well washed with water and separated by means of a centrifuge. The final bacterial suspension contained 0·0785 grm. dry weight of cocci in 1 c.c.; 2 c.c. of this suspension were added to a series of tubes each containing 6 c.c. of an emulsion of tar acids, ranging in strength from 0·15 to 0·89$^0/_0$. The contents were well mixed and, after two hours, centrifuged. A measured quantity of the supernatant liquid was removed and acidified, the tar acids extracted with ether, and determined (see this *Journ.* p. 683).

The results are set out in Table II, and show that the emulsified tar acids removed by one gramme of staphylococci is at first roughly proportional to the original concentration of tar acids, but, as this concentration increases, its influence becomes progressively less and the amount removed rapidly tends to a maximum.

TABLE II.

Adsorption of emulsified tar acids by bacteria.

	Organism employed	Concentration of bacteria dry weight $^0/_0$	Initial concentration of tar-acids $^0/_0$	Final concentration of tar-acids $^0/_0$	Amoun adsorbed by 1 grm. dry weight bacteria, grms.
Exp. 19. 9. '08	Morgan's	1	0·714	0·414	0·300
	Bacillus	1	0·178	0·094	0·084
Exp. 28. 9. '08	*Staphylococcus*	1	0·892	0·496	0·396
	pyogenes aureus	1	0·594	0·228	0·366
		1	0·446	0·164	0·282
		1	0·298	0·086	0·212
		1	0·148	0·072	0·076

The results with bacteria are indeed similar to those obtained by us with animal charcoal (Table XI, this *Journ.* p. 684), but one gramme dry weight of bacteria removes, under similar conditions, about twice as much of the emulsion as was removed by the preparation of animal charcoal employed, viz. 39·6 % of its own weight.

We found that the cocci lost about 50 % of water on drying at 110° C. so that weight for weight bacteria and animal charcoal happen to be about equally effective. This similarity between the two processes is strikingly demonstrated if the figures for the amounts removed by one gramme of bacteria and charcoal respectively are both plotted against the original concentration of the emulsion. This has been done in Fig. 1, where the circles represent the amounts of tar acids adsorbed by one gramme charcoal and the crosses that removed by one gramme of bacteria in their natural condition. It will be seen that the curve drawn for the adsorption by charcoal would do almost equally well for that by the cocci.

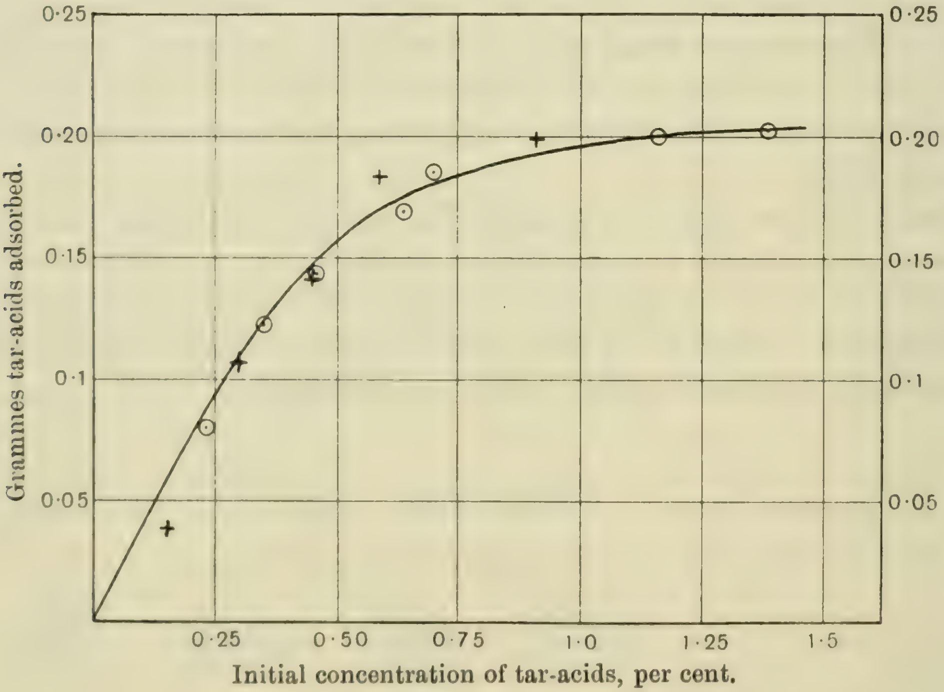

Fig. 1.

From these results there is little doubt that the removal of an emulsion of tar acids by bacteria is, in the first instance, a process of adsorption and not a chemical combination, and that disinfectants of this class possess superior efficiency, because owing to this adsorption the bacteria rapidly become surrounded by the disinfectant in much greater concentration than exists throughout the liquid. As has been pointed out elsewhere, however (this *Journ.* p. 689), the same property of adsorbing the particles of the emulsion is possessed by most organic particles, in consequence of which the germicidal value of this class of disinfectant is greatly deteriorated by the presence of particulate organic matter other than the bacteria which it is desired to destroy.

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OBSERVATIONS INDICATING THAT THE RECENT OUTBREAK OF DIPHTHERIA IN THE WOOD-PIGEON (*COLUMBA PALUMBUS*) IS CAUSED BY A "FILTER-PASSER."[1]

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The great majority of those who have studied diphtheria in pigeons and other fowls are agreed that bacteriologically it is quite distinct from human diphtheria, and cases where they have been regarded as identical have not been based upon bacteriological identification.

Various bacteria have been described as associated with diphtheria in birds.

Loeffler (1884) described a non-motile gram-negative bacillus in pigeon diphtheria, and called it *Bacillus diphtheriœ columbarum*. By inoculating the scarified throat of a healthy pigeon with this bacillus he reproduced the disease, which proved fatal in one to three weeks; when injected into the muscles it produced œdema and then suppuration, but was not fatal. It was also pathogenic for mice and rabbits, but the hen was resistant. Loeffler's results have been confirmed by other observers. In another epizootic, Haushalter (1891), Loir and Ducloux (1894), and Quaranta (1897), on the other hand, found a gram-negative motile bacillus which also reproduced the disease and affected all birds; subcutaneous injection with this bacillus produced lesions similar to those found in the pharynx. Moore (1895), investigating an epidemic in America, associated the outbreak with a *Pasteurella* identical with that of chicken cholera and swine-plague. Harrison and Streit (1903) associated the disease with the presence of two bacilli, the *B. pyocyaneus* and the *B. cacosmus.* Streit (1904), in a very exhaustive study of this disease, found, as before, *B. pyocyaneus* and another bacillus which he called the "Roup bacillus." The latter was an actively motile gram-negative bacillus which, when grown on broth, produced, in addition to the general turbidity, a fine membrane, easily broken up: it produced gas, with slight acidity, on glucose media, it clotted milk, but produced no change in lactose and cane sugar. He also described in the bronchial exudate, protozoiform bodies consisting of swollen epithelial cells with a transparent body in the centre. Guérin (1902–3) isolated a cocco-bacillus with which he reproduced the disease; he also raised its virulence by conjunctival inoculation, and was able to immunise animals with a specific antiserum.

Reiner Müller (1906) isolated a bacillus which morphologically, though not biologically, resembled the Klebs-Loeffler diphtheria bacillus. It grew in

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[1] Received June 25, 1908.

pure culture only on blood agar media, and each colony was surrounded by a clear halo. He found that this bacillus grew on ordinary agar in symbiosis, and stated that, on growing it with a sarcina in broth and then filtering, the filtrate behaved in a similar manner ("die in gleicher Weise wirkte") to the living bacillus. Many observers, including M'Fadyen and Hewlett (1899), Gallez (1895), Ferré (1898), Gratia and Liénaux (1898), Gordon Sharp (1900), Jenner (1900), Harrison, and Guérin, have also described in this disease the presence of a micro-organism morphologically and culturally resembling the Klebs-Loeffler bacillus of diphtheria, but when bacteriologically tested these proved to be non-virulent. Graham-Smith (1904) isolated a similar bacillus from the throat of a healthy fowl.

Flagellata and other protozoa have also been described in this disease.

Present Investigation.—During an outbreak of pigeon diphtheria occurring in this country in the early part of this year, the bodies of eight wood-pigeons were sent to the Institute for examination. Seven of these were sent in the beginning of February and one in the middle of March.

The post-mortem examinations in all these cases showed identical pathological conditions. These consisted in the presence of a yellow cheesy membrane, usually in a dense mass, in the pharynx, and extending, in isolated colonies, down the œsophagus to the crop. The trachea was not involved in any of these cases. On section the membrane was found to consist chiefly of leucocytes, red blood corpuscles, broken-down epithelial cells and numerous micro-organisms. The latter consisted of bacilli and cocci, which were scattered throughout, but occurred for the most part in small clumps. Smears made from the membrane showed the presence of epithelial cells, leucocytes and numerous micro-organisms, but none of the protozoiform bodies described by Streit were found.

The bacteria were isolated by plating on ordinary agar plates. Altogether ten different micro-organisms were isolated from the membrane. The morphology and fermentation characteristics of these were examined, but as cultures of all of them failed to produce the disease, these particulars will not be considered in the present note.

Having obtained negative results with the cultures of the organisms isolated from the throat of the affected pigeons, we tried inoculating the scarified throats of healthy doves with pieces of the membrane. This also failed to infect.

In another experiment the membrane from a pigeon dead of the disease was emulsified in broth, filtered through a Berkefeld filter, and the throat of a healthy dove (No. 20) swabbed with the filtrate on February 4. The filtrate proved on subculture on various media to be sterile. Three weeks later, February 26, dove No. 20 became ill, had great difficulty in swallowing, and died on February 27, 1908. The lesions produced were typical of the disease, the membrane extending all over the pharynx and spreading down the œsophagus.

The throats of two doves, 21 and 22, were inoculated with the filtrate from the membrane of dove No. 20 : one died soon after inoculation without any ascertainable cause, and the other gave negative results. Another dove, No. 23, was injected subcutaneously with 1 c.c. of the filtrate from dove 20, and died four days after inoculation without any apparent pathological changes. One c.c. of the citrated blood of this dove (No. 23) also proved fatal in two days when injected subcutaneously into another dove (No. 24). One c.c. of the citrated blood from dove 24 was injected subcutaneously into dove 25. No. 25 died after a long interval on April 30, 1908. Neither in dove 24 nor in dove 25 could any pathological condition be found on post-mortem examination. The heart blood in doves 23 and 24 proved sterile on sowing on ordinary agar.

Unfortunately, by the time the above experiments were completed the epizootic had ceased, and no more material was available. It appears, however, that an extract of the membrane after filtration through a Berkefeld filter still contained the virus, and that this reproduced the complete picture of the disease when inoculated upon the throat (dove 20); whereas, when subcutaneously inoculated, an acutely fatal disease without any definite pathological lesions was produced (dove 23). The blood of a pigeon which had succumbed to the acute infection by the filtrate (No. 23), although sterile when cultivated upon agar, occasioned an acute infection, and death when 1 c.c. was injected subcutaneously into another pigeon (No. 24). The disease was carried on through two further generations of pigeons by inoculation of the heart blood. The last pigeon, however, did not die acutely, and its blood failed to infect.

Molluscum contagiosum, which is a well-recognised disease affecting fowls, has been proved by Marx and Sticker (1902, 3) to be due to the presence of a filter-passer. It is interesting to note in this connection that Carnwath (1907), working with diphtheria in hens, obtained the following results. He failed to reproduce the disease with cultures from the diphtheritic lesions, but noticed in one of the hens a small isolated wart in the mouth which he thought might be molluscum contagiosum, and with this he produced typical molluscum or fowl-pox lesions on the comb and other susceptible parts. Also by inoculating the throat of a healthy hen with the filtrate from these molluscum lesions he produced typical fowl-diphtheritic lesions in the pharynx. He could not convey the disease from the hen to the pigeon. He concluded that these two diseases had probably a common causative factor. Lipschütz (1908), investigating molluscum contagiosum in pigeons, also got negative results by cultural methods, but so far had not succeeded in reproducing, with the molluscum virus, lesions resembling typical pigeon diphtheria. Though in some cases we found isolated colonies of membrane in the œsophagus of the pigeon, their appearance did not suggest to us that the disease was molluscum contagiosum. Only, the presence of a filter-passer, the negative results with cultures, and the work of the above-mentioned observers suggested the possibility.

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SOME EXPERIMENTS ON IMMUNITY AGAINST VACCINIA IN ANIMALS.

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Hitherto all efforts to set up immunity against vaccinia in animals have been restricted to the vaccination of animals in the customary manner, or to the injection of vaccine lymph into them.

In the present series of experiments an attempt was made to derive from calf vaccine an unorganised body capable of causing specific immunity against vaccinia in animals.

The method of production of such an immunisator has been :—

1. Collection of vaccine pulp from a calf.
2. Trituration of this pulp with normal saline solution.
3. Heating this mixture at 60° C. for 1 hour, in some instances as noted below.
4. Storage of this mixture.
5. Filtration of this mixture.

In the first experiment guinea-pigs were used. Into these animals filtrates of autolysed lymph were injected subcutaneously as detailed in the following Table I. Six varieties of filtrates were prepared from the pulp of one calf as follows:—

(*a*) *Unheated portion of mixture.*

1. Passed through a Berkefeld filter.
2. „ „ Chamberland filter.
3. „ „ Martin's gelatin filter.

(*b*) *Heated portion of mixture.*

4. Passed through a Berkefeld filter.
5. „ „ Chamberland filter.
6. „ „ Martin's gelatin filter.

Each of these six filtrates was injected subcutaneously at intervals into a series of eight pigs. Subsequently these pigs were vaccinated in the usual manner with one strain of lymph, as were eight control pigs which had received no injection; and the result of such vaccination was noted daily for a week.

The following Table I shows the results obtained.

TABLE I. (8 *guinea-pigs in each cage.*)

+ = Specific vesiculation. ? = Specific reaction not amounting to vesiculation. – = No specific reaction.

No. of cage	Material injected	Injections April 4	April 22	April 29	Date of vaccination	Result of vaccination
23	Nil (control pigs)	nil	nil	nil	May 6	8+
25	Berkefeld V filtrate of heated autolysed lymph	1 c.c.	1 c.c.	4 c.c.	,,	7– 1 ?
26	Chamberland filtrate of heated autolysed lymph	,,	,,	,,	,,	5– 3 ?
27	Martin's gelatin filtrate of heated autolysed lymph	,,	,,	,,	,,	6+ 2–
28	Berkefeld V filtrate of unheated autolysed lymph	,,	,,	,,	,,	6– 2+
12	Chamberland filtrate of unheated autolysed lymph	,,	,,	,,	,,	6– 2+
22	Martin's gelatin filtrate of unheated autolysed lymph	,,	,,	,,	,,	6+ 2–

The results obtained with the control pigs differed markedly from those which had received subcutaneous injections of Berkefeld and Chamberland filtrates in that while the controls all developed good vesicles, the others consistently failed to develop vesicles, and only occasionally showed some modified specific reaction. Again, the pigs injected with Martin's gelatin filtrates as a rule showed vesiculation though possibly some immunisation had been set up by these filtrates. The immunisation set up by heated autolysed filtrates seemed on the whole to be better than that set up by unheated ones.

Experiment 2 was made on the same lines as the foregoing with the results detailed in Table II.

The results obtained by this second experiment bring out, if anything, more clearly, the points shown by experiment 1.

Experiment 3. A calf was injected subcutaneously with a Berkefeld filtrate of a heated autolysed vaccine. On July 10th, 10 c.c. were injected; on July 23rd, 10 c.c.; and on July 30th, 15 c.c. On August 7th the calf was vaccinated. No vesiculation resulted. Good vesiculation occurred on a control calf vaccinated with the same lymph.

TABLE II. (8 *guinea-pigs in each cage.*)

+ = Specific vesiculation. ? = Specific reaction not amounting to vesiculation. – = No specific reaction.

No. of cage	Material injected	Injections May 13	 June 4	 June 11	Date of vaccination	Result of vaccination
23	Nil (control pigs)	nil	nil	nil	July 8	8+
25	Berkefeld V filtrate of heated autolysed lymph	2 c.c.	2 c.c.	2 c.c.	,,	7– 1?
26	Chamberland filtrate of heated autolysed lymph	,,	,,	,,	,,	6– 2?
27	Martin's gelatin filtrate of heated autolysed lymph	,,	,,	,,	,,	5+ 1? 2–
28	Berkefeld V filtrate of unheated autolysed lymph	,,	,,	,,	,,	7– 1?
12	Chamberland filtrate of unheated autolysed lymph	,,	,,	,,	,,	7– 1+
22	Martin's gelatin filtrate of unheated autolysed lymph	,,	,,	,,	,,	4+ 2? 2–

Experiment 4. A monkey was injected with a Berkefeld filtrate of heated autolysed vaccine. Subcutaneous injections were given:— November 25th, 10 c.c.; November 27th, 10 c.c.; November 30th, 10 c.c. On December 6th the monkey was vaccinated on a shaved area over the scapulae in linear incisions. On December 13th, the eighth day after vaccination when vesiculation should be at its height, two or three small round abortive vesicles, each about the size of a split pea, were visible at intervals along the lines of incision. On December 10th these vesicles had dried up. Proliferation of tissue forming a heaped up crust along each incision followed, but no further vesiculation.

Experiment 5. A monkey was injected subcutaneously with a Berkefeld filtrate of autolysed heated vaccine. On Jan. 20th, 15 c.c. were injected; on Jan. 23rd, 30 c.c.; on Jan. 28th, 15 c.c.; and on Jan. 31st, 30 c.c. On Feb. 13th the monkey was vaccinated. On Feb. 20th a few small abortive vesicles were visible as in experiment 4, along the lines of incision, but these were even less marked than those in experiment 4.

The evidence afforded by this limited number of experiments points to the facts:—

1. That it is possible to produce from calf vaccine a specific unorganised immunisator.
2. That this immunisator is of such a nature as to be capable of filtration through a Berkefeld V or a Chamberland filter, and possibly in some small degree through a Martin's gelatin filter.

The fact that such an immunisator can be obtained, naturally suggests that it might be capable of setting up immunity against small-pox. It has not yet been found possible, however, owing to the scarcity of small-pox material, to carry out any work on these lines. Experiments will be made at the first opportunity. It is evident that if an immunisator against small-pox could be prepared from calf-vaccine its use would lead, in all probability, to an appreciable gain in time in dealing with small-pox contacts. Usually, of course, such contacts are vaccinated, and when the vaccination is successful an immunisator is manufactured in the vaccinated area of the patient, which immunisator is absorbed and gives rise to protection. If, however, the immunisator could be given directly to the contact, the time necessary for him to manufacture his own immunisator would be saved, and such gain in time might be important. Furthermore, the injection of the immunisator could, if desired, be used in addition to, and not instead of, vaccination in these cases. The immunisator might also possibly be injected advantageously during the course of small-pox. Until investigation on these lines is possible, other points in connection with the behaviour of the immunisator are being worked at.

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THE INFLUENCE OF TEMPERATURE, AND SOME OTHER PHYSICAL CONDITIONS, ON CALF VACCINE.

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1. Temperature.

The effect of temperature on calf vaccine is a matter both of scientific interest and of economic importance in preventive medicine. The latter point is of especial significance in hot climates, where rapid deterioration of the potency of lymph is not uncommon; whilst, irrespective of latitude, knowledge of the optimum temperature at which vaccine lymph can be stored is much to be desired.

Work on these lines with vaccine has been done by Blaxall (1902), Blaxall and Fremlin (1906), and Carini (1906); and with vaccine and various organisms by Macfadyen and Rowland (1900).

It seemed desirable, however, to extend the scheme of work as regards calf vaccine adopted by these observers, and to some extent to amend them. It seemed worth while, for instance, instead of exposing one vaccine in the form of dry powder at 60° C., and another as glycerinated suspension at 10° C., and so on, to take for each experiment vaccine collected from one calf; to prepare this in a number of ways, and to ascertain the behaviour of these preparations under different temperature conditions.

In the present experiments vaccine from one calf has been used in the following forms:—

(*a*) *Crude exuded lymph.* This is the exudate expressed from vesicles 120 hours after vaccination of the calf, stored in sealed capillary tubes. This lymph generally coagulates soon after collection, the coagulum adhering to the sides of the tube. The fluid portion, separated from the coagulum, sealed up in capillary tubes was used for experiment.

TABLE I.

+ = Vesiculation. ? = Specific reaction not amounting to vesiculation. – = Absence of specific reaction.

No. of Experiment	Preparation of vaccine	59—60° C. Minutes							36—37° C. Days					10—15° C. Weeks					3—4° C. Weeks					–3—–4° C. Weeks	
		1	5	10	15	20	30	60	1	5	8	12	20	4	8	12	25	52	4	8	12	25	52	25	52
1	Exudate	+	+	+	+	?	–	–	+	+	+	+	+	+	+	+	+	?	+	+	+	+	?	+	+
	Glycerinated	+	+	+	?	–	–	–	+	+	+	+	?	+	+	+	?	–	+	+	+	+	?	+	+
	Chloroformed	+	+	+	?	–	–	–	+	+	+	?	?	+	+	+	+	–	+	+	+	+	?	+	+
	Dry powdered	+	+	+	+	+	+	?	+	+	+	+	+	+	+	+	+	–	+	+	+	+	?	+	+
	Lanolinated	+	+	+	+	+	+	?	+	+	+	+	+	+	+	+	+	–	+	+	+	+	?	+	+
2	Exudate	+	+	+	?	–	–	–	+	+	+	+	?	+	+	+	+	?	+	+	+	+	+	+	+
	Glycerinated	+	+	–	–	–	–	–	+	+	+	–	–	+	+	+	–	–	+	+	+	+	?	+	+
	Chloroformed	+	+	?	–	–	–	–	+	+	+	?	–	+	+	+	?	–	+	+	+	+	?	+	+
	Dry powdered	+	+	+	+	+	+	–	+	+	+	+	–	+	+	+	?	–	+	+	+	?	–	+	–
	Lanolinated	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+	+	+
3	Exudate	+	+	+	+	–	–	–	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+	+	+
	Glycerinated	+	+	+	–	–	–	–	+	+	+	–	–	+	+	+	+	–	+	+	+	+	?	+	+
	Chloroformed	+	+	?	–	–	–	–	+	+	+	–	–	+	+	+	+	–	+	+	+	+	?	+	+
	Dry powdered	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	?	+	–
	Lanolinated	+	+	+	+	+	+	?	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+	+	+
4	Exudate	+	+	+	–	–	–	–	+	+	+	+	?	+	+	+	+	–	+	+	+	+	+	+	+
	Glycerinated	+	+	–	–	–	–	–	+	+	?	–	–	+	+	+	+	–	+	+	+	+	?	+	+
	Chloroformed	+	+	?	–	–	–	–	+	+	–	–	–	+	+	+	+	–	+	+	+	+	?	+	+
	Dry powdered	+	+	+	+	+	?	?	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	–
	Lanolinated	+	+	+	+	?	–	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+
5	Exudate	+	+	+	+	–	–	–	+	+	+	+	?	+	+	+	+	–	+	+	+	+	?	+	+
	Glycerinated	+	+	+	–	–	–	–	+	+	+	?	–	+	+	+	?	–	+	+	+	+	?	+	+
	Chloroformed	+	+	+	–	–	–	–	+	+	+	+	?	+	+	+	?	–	+	+	+	+	?	+	+
	Dry powdered	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	–	+	?
	Lanolinated	+	+	+	+	+	?	–	+	+	+	+	+	+	+	+	+	–	+	+	+	+	?	+	+

(*b*) *Glycerinated calf vaccine.* This is a suspension of one part by weight of finely triturated vaccine pulp in four parts by weight of a 50 % glycerine and water solution.

(*c*) *Chloroformed calf vaccine.* This is a suspension of one part by weight of finely triturated vaccine pulp in two parts by weight of distilled water, subjected to the action of chloroform vapour and air for from 10 to 15 minutes at 20° C. with subsequent removal of all chloroform and the addition of two parts by weight of glycerine.

(*d*) *Desiccated calf vaccine.* This is vaccine pulp reduced to dry powder.

(*e*) *Lanolinated calf vaccine.* This is a mixture of one part by weight of calf vaccine pulp with four parts by weight of lanolin.

All these were placed in sealed capillary glass tubes except (*e*) which, on account of its consistency, had to be placed in a wider-mouthed vessel of about 0·25 c.c. capacity which was hermetically sealed.

These tubes were placed at 59°—60° C., 36°—37° C., 10°—15° C., 3°—4° C. and – 4° C. for varying intervals of time. The contents of the tubes were inoculated on a calf 24 hours after the exposures had finished, and the results of such inoculations were noted 120 hours later.

The results obtained in these experiments are shown in Table I.

Table II is obtained by an analysis of the results in Table I, and gives the average result of the five experiments, and shows that whereas glycerinated lymph, whether treated previously with chloroform or not, retained its infectivity in a very similar manner under the various conditions of temperature; the other three varieties of material behaved somewhat differently.

TABLE II.

Preparation of vaccine	Average duration of infectivity at different temperatures				
	60° C. Minutes	37° C. Days	10—15° C. Days	4° C. Days	– 4° C. Days
Glycerinated	11	12	203	364	>364
Chloroformed	11·5	13	224	364	>364
Exudate	16	20	322	>364	>364
Lanolinated	39	20	301	>364	>364
Dry powdered	51	>20	252	287	301

I am indebted to Dr C. J. Martin for pointing out to me that the results in Table II indicate a certain regularity in the effect of temperature upon the durability of calf lymph. For example if logarithms of times as ordinates be plotted against degrees of temperature as abscissae, the results of the experiments form points on a straight line (Fig. 1).

In other words there exists a regular logarithmic relation between the duration of infectivity and the temperature at which the lymph is kept, which is expressed by the equation

$$\frac{1}{T_2 - T_1} \log \frac{t_1}{t_2} = \text{a constant},$$

where t_1 and t_2 represent the time of durability of lymph at temperatures T_1 and T_2 respectively.

From the above observations the temperature coefficient for the velocity with which glycerinated lymph deteriorates can be determined and is found to be about 3 for each 10°, so that lymph kept at 20° C. deteriorates three times as quickly as lymph kept at 10° C.

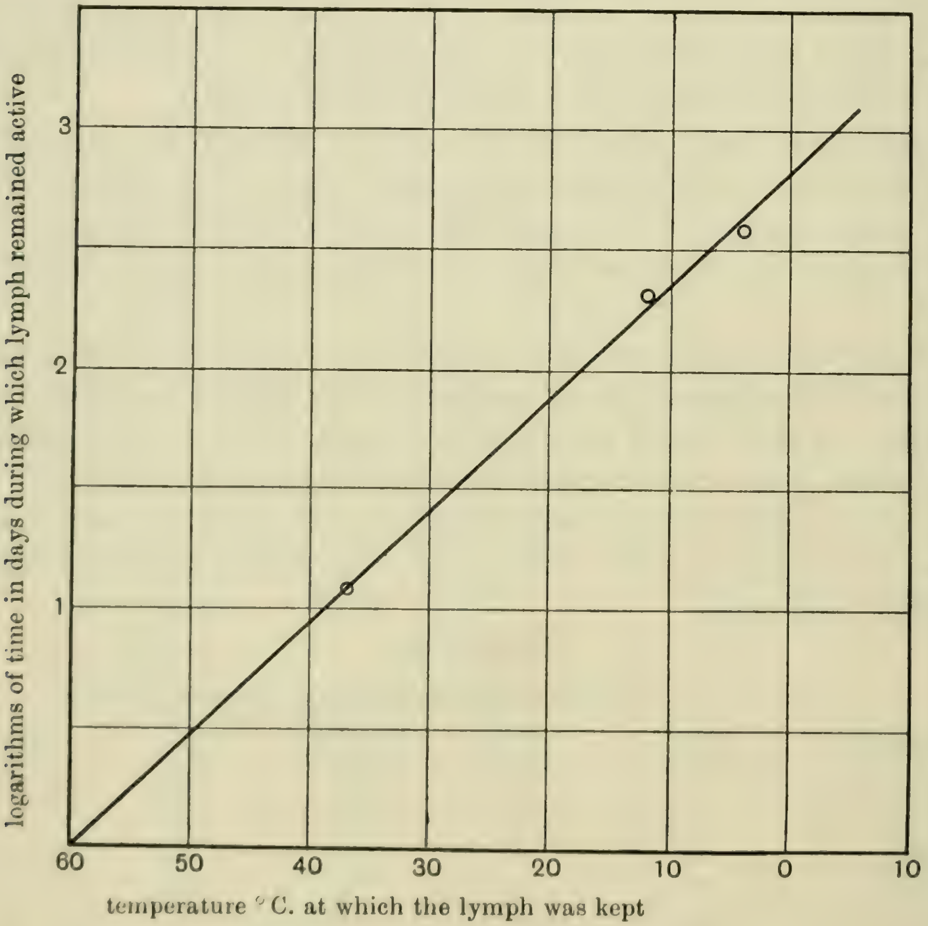

Fig. 1.

A point of interest is that the effect of temperature in hastening the deterioration of glycerinated lymph obeys the same law as is followed by an ordinary chemical reaction and the effect is moreover quantitatively of the same order, viz. two to three times for a rise of 10° C. A similar law for the influence of temperature upon the germicidal action of mercuric chloride and other disinfectants has been found by Madsen and Nyman (1907) and H. Chick (1908) to apply in the case of anthrax spores and vegetative forms of bacteria.

This logarithmic law is obeyed up to the temperature of 37° C. If however from the data below and at this temperature, the time of durability at 60° C. is calculated from the above formula, it is found to be about one day, or one hundred times that found by experiment. Somewhere between 37° and 60° temperature *per se* begins to influence the vitality of the lymph unfavourably. If Fig. 1 is reconstructed, plotting logarithms of velocities of destruction of lymph (reciprocals of durability) as ordinates against temperature as abscissae, the points obtained for temperatures 4° C., 12·5° C., 37° C. lie nearly on a straight line, but the point corresponding to the temperature of 60° C. is reached by making the slope much steeper. This indicates that an added effect due to temperature alone, is, somewhere between 37° C. and 60° C., superposed upon the gradual loss of vitality which proceeds at all temperatures.

A practical advantage which follows from the discovery of this regular temperature coefficient for this process of destruction of the virus in calf lymph is that one can foretell the results of experiments which are in themselves very tedious to make. For example the length of durability at – 4° C. was found to exceed one year but was not determined in any instance (see Tables I and II), owing to the extreme slowness of the process of destruction at that temperature. By an application of the above formula it appears that at a temperature of about – 4° C. glycerinated lymph would still be active for nearly three years (1000 days). This figure can be obtained also by continuing the straight line drawn in Fig. 1, until the logarithm of the corresponding time can be read off for a temperature of – 4° C. (log time at this point = about 3·00 and time = 1000).

In some further experiments portions of lymph pulp derived from six calves 120 hours after vaccination were dried, powdered and sealed up in glass capillary tubes. In order to ascertain the limit of resistance of the potency of dried vaccine to a temperature of 99° to 100° C., these capillary tubes were immersed in boiling water, one tube of each of the six experimental lymphs being kept at room temperature as a control. A few hours after such exposure these lymphs were inoculated on a calf, and the results noted 120 hours later.

These results are shown in the following table III.

TABLE III.

+ = Vesiculation. ? = Specific reaction not amounting to vesiculation.
– = Absence of vesiculation.

Number of experiment	Potency of control	Potency of dried powdered calf vaccine in sealed capillary tubes after immersion in boiling water Minutes									
		1	2	3	4	5	10	15	20	30	60
1	+	+	+	+	+	+	?	–	–	–	–
2	+	+	+	+	+	+	–	–	–	–	–
3	+	+	+	+	+	+	–	–	–	–	–
4	+	+	+	+	+	+	–	–	–	–	–
5	+	+	+	+	+	+	+	?	?	–	–
6	+	+	+	+	+	+	?	–	–	–	–

That is to say, dried powdered calf vaccine after exposure in the manner described to a temperature of 99° to 100° C. gave rise to vesiculation in every case after an exposure of five minutes, in one case after exposure for 10 minutes, and in one case showed distinct signs of specific reaction by marked induration and redness along the line of inoculation after exposure for 20 minutes.

Expressed calf lymph and lanolinated calf lymph have shown no signs of potency after exposure for one minute to a temperature of 99° to 100° C.

This resistance of dried calf vaccine to a temperature of 99° to 100° C. has apparently not been investigated before, and I am indebted to Dr C. J. Martin for suggesting it.

2. Osmotic pressure.

Negative osmosis.

It is well known that the specific virus of vaccinia is able to withstand for many weeks association with either pure glycerine or with a solution of glycerine in distilled water in various proportions.

The result of such association is very slight progressive loss of potency on the part of the specific virus.

Similarly, association of calf vaccine with a saturated watery solution of some of the sugars, notably cane sugar, results in but slight loss of potency to the vaccine.

From some experiments which I have made, this resistance is shown to be as marked at room temperature when freshly expressed fluid vaccine is admixed with either glycerine or with a saturated watery solution of cane sugar, as when calf pulp is admixed with these substances.

This persistence of pathogenicity implies marked ability of the vaccine virus to withstand negative osmotic pressure. For one of the chief characteristics of both glycerine and of cane sugar is their hygroscopic influence.

Positive osmosis.

Novy and Knapp (1906) found that *Tr. brucei* and *Tr. lewisi* and *Sp. obermeieri* in rats' blood were affected injuriously in varying degrees by dialysis against running distilled water for different lengths of time, extending from a few minutes to several hours.

Following on Novy and Knapp's work Ballah (1906) described some experiments on calf vaccine and concluded that the potency of vaccine was not affected by dialysis against distilled water for 18 hours. I have been able to confirm Ballah's statements.

Ballah's work, however, differed in technique from that of Novy and Knapp in an important particular, on which Ballah makes no comment. For, whereas Novy and Knapp placed blood containing their organisms in the dialyser, Ballah dialysed "vaccine emulsion." If this emulsion was a glycerinated suspension, as must be concluded in the absence of a definite statement to the contrary, then Ballah probably subjected vaccine organisms to positive osmotic pressure after they had been already subjected to negative osmotic pressure by glycerine.

It was with a view to the avoidance of such a complication that the lymphs of the present series of experiments were selected in a condition as analogous as possible to the bloods of Novy and Knapp.

In the present series vaccine lymph was expressed from the vesicles of a calf 120 hours after vaccination.

This lymph was collected in glass capillary tubes which were then sealed, and coagulation was then allowed to occur. After the coagulum had shrunk somewhat—20 hours after collection—the fluid contents of the tubes were removed to fine collodion sacks. These sacks were sealed, and were suspended in running distilled water for from 2 to 24 hours at room temperature, and the potency of their contents was subsequently compared on calves with that of a control (undialysed) portion of each vaccine.

Out of a series of 37 experiments no evidence was obtained to show that dialysis had any injurious action on the specific vaccine virus.

3. FILTRATION.

During the past few years renewed attempts have been made to investigate the filterability of calf vaccine. Earlier efforts pointed to the inability of the vaccine virus to pass through bacteriological filters. But in 1905 Negri published an article which indicated that, under certain conditions, the virus was capable of passage through a Berkefeld filter. The chief difference between the technique of Negri and that of former investigators lay in the fact that Negri worked with vaccine emulsion, which, after trituration to a degree of extreme fineness, had been stored in the cold for two weeks or more after preparation. The theory advanced for the filterability of vaccine organisms of such an emulsion was that this storage led to changes in the size of the organism, analogous perhaps to the formation of spores, whereby they could pass through the filter channels while the larger sized organisms could not. Previous investigators would appear to have worked with coarser and more recent emulsions.

Remlinger and Nouri (1905), and Siegel (1905), published papers in 1905 confirming Negri's results.

In 1906 Carini (1906) published his work on the subject with a carefully reasoned argument. This last named investigator appears to think that, working on the lines laid down by Negri, a very small number of specific organisms may pass through Berkefeld V and N filters. He is however careful to mention (*a*) that his experimental inoculations of these filtrates were made on animals which were used simultaneously for the routine production of vaccine, and therefore, that accidental contamination of his experimental incisions could not be absolutely excluded; and (*b*) that the character of such vesicles as he obtained was poor, and indicated inoculation with a degenerated virus. It is important to note, though Carini does not point it out, that vesicles of this type might easily result when a partly dried incision is insufficiently inoculated with normal vaccine; such a condition in fact as might be afforded were a minute quantity of vaccine from a normally vaccinated surface to be carried into another incised, but previously uninoculated, surface of the same animal.

During the last two years I have carried out a number of experiments on the filtration of calf vaccine emulsion. During the earlier part of this work I was unacquainted with Negri's procedure, but after the publication of his technique great care was taken to follow this closely in the case of over forty experiments. In many cases the filtrates were centrifugalised and the upper layers were pipetted off to try and concentrate any organisms which might be present.

All the filters were Berkefeld's N or V. These were new filters of the smallest laboratory pattern, sterilised before use.

In order to test the filters for possible defects, emulsions were examined, previous to passage, for extraneous micro-organisms and the filtrates were examined subsequently to ascertain whether these had passed through, and if so, in what quantity.

The filtrates were inoculated in some instances on guinea-pigs and in all cases on calves. A control, or unfiltered portion of emulsion, in each instance was inoculated on the same animal. The calves were used at the same time for the routine production of vaccine.

Of the results of the inoculations on guinea-pigs every one was negative, while the controls showed specific activity.

Of the results on calves every filtrate, with two exceptions, gave a negative result; while the controls on the same animals gave well-marked vesiculation.

In the case of the first of the two exceptions distinct vesiculation showed 120 hours after inoculation. The site of the inoculation was on the inner side of the right hind leg, about 5 cm. from the ilio-inguinal fold and at right angles to it. The inoculation was a line about 5 cm. long. The site in question had been ill-chosen for an experimental inoculation, for when the calf was standing up this surface of skin was in contact with a portion of a surface of skin which was being used for routine vaccination; although when the calf was on the vaccination-table the two surfaces were separated considerably.

In this case, the possibility that this experimental incision was inoculated accidentally from the skin surface with which it came in contact, cannot be excluded.

In the case of the second exception there was no question of direct contact between the experimental incision and the normally vaccinated surface. But at the site of inoculation, 120 hours after inoculation with a filtrate of a Berkefeld V filter, there was one spot of an apparent attempt at vesiculation tailing off into a slightly raised red papule. The appearance in fact was that of very feeble specific reaction. The control of the filtrate produced well-marked vesiculation, though not of first-rate quality, on the same animal.

Apart from the proximity of the control the whole abdomen of the calf was used for routine vaccination as well as a small portion of the thorax somewhat adjacent to the site of the experimental incision, and it is impossible therefore to exclude entirely the possibility of accidental contamination, though it is quite possible that no such contamination occurred.

The results of these experiments as a whole cannot be said to afford confirmation of Negri's work. On the other hand they appear to be parallel with Carini's results, and to show that if the specific virus of vaccinia is capable of passage through a Berkefeld V such passage is of rare occurrence, and is limited to a very small number of germs.

It has been stated earlier that my first experiments were not carried out according to Negri's technique. With these a 1 in 5 emulsion was usually filtered through a Berkefeld V or N, and occasionally through a Pasteur-Chamberland by means of a partial vacuum, without a preliminary passage through filter paper or cotton wool. A result was then commonly obtained which I have not seen noted elsewhere.

When the filtrate was examined, after negative pressure had been going on for an hour or more, small white masses of organic matter were noticed, and often in considerable amount. These masses were not uncommonly so large as to point to a defect in the filter. But *B. subtilis* and staphylococci failed to pass through the filter, while the white particles were present in the filtrate in numerous large fragments.

With fractional filtration it was noted that while all fractions might be clear for some minutes after passage, they gradually became clouded and the white precipitate appeared, the fragments of which increased in size for a time.

This precipitate appeared in the filtrates of Berkefeld's V or N, to a lesser extent in the filtrate of a Pasteur-Chamberland, and not at all in the filtrates of a Martin gelatin filter. The precipitate generally appeared least dense in the earliest fractions.

Hitherto this precipitate, separated from the bulk of the fluid filtrate by centrifugalisation, has failed to give any specific lesions on calves, while the control (unfiltered portion of the same vaccine) has yielded typical vesicles.

In view of this fact, if for no other reason, the microscopical appearance of the precipitate is of interest. I hesitate at the present time to say that any of the microscopic elements of the precipitate resemble definitely the body or bodies described as the specific cause of vaccinia by authors, although it seems to me that in a hanging drop preparation they are not unlike some published descriptions of the vaccine organism.

SUMMARY OF CONCLUSIONS.

1. Temperature influences the rate at which glycerinated lymph deteriorates in accordance with the law of Arrhenius, expressing the effect of temperature upon chemical action; the rate of deterioration increasing three times per 10° rise in temperature.

2. Dry powdered calf vaccine in sealed glass capillary tubes still gave rise to typical vesicles after exposure to a temperature of 100° C. for from 5 to 10 minutes.

3. The vaccine virus can withstand positive osmosis for 24 hours or more, and negative osmosis for 8 weeks or more, at room temperature.

4. It appears doubtful whether the vaccine virus will pass through a Berkefeld V filter even when a stored vaccine emulsion is used for filtration. If any virus does pass through it only does so exceptionally and in small quantities.

5. When a glycerinated vaccine emulsion is filtered, white flocculi frequently form in the filtrate. These are at first minute, but subsequently adhere and form a macroscopic sediment. This sediment, when inoculated on calves, does not cause vesiculation.

Microscopically these flocculi, in hanging drop preparations, resemble the vaccine bodies described by one or more observers.

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N° 11

AN OUTBREAK OF COW-POX.

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THE following is of interest inasmuch as it is a record of a somewhat unusual occurrence—namely, an outbreak of cow-pox in a herd of milch cows. The herd is the property of a farmer in Hertfordshire.

During the middle of March of the present year it was noticed that the teats of several of this herd of cows were cracked or "chapped." This was attributed by the farmer to the east winds which were prevalent at the time, and no importance was attached to it as similar lesions are not infrequent among cows in the early spring. About two weeks after these cracks appeared definite sore places began to develop on the same areas. 25 out of a herd of 27 cows were affected in this way. On April 6th the farmer, a man of about 50 years of age, noticed sore places on both his hands. Both hands became severely inflamed, in consequence of which he consulted Dr. Daniel Wilson of Bushey Heath. The right hand was more affected than the left. Dr. Wilson found papular areas on the fingers and palmar and dorsal surface of the right hand, and one or two papules on the left hand, accompanied by extensive œdema of the surrounding subcutaneous tissues and redness of the neighbouring skin. The axillary glands were also enlarged. A day or two later one or two similar papules developed on the patient's forehead and one on the upper lip accompanied by local swelling and redness and considerable constitutional disturbance. Three or four days later all the papules developed a vesicular stage, the vesicles showing umbilication and presenting the appearance of vaccine vesicles of a severe type. A clear fluid resembling the discharge of vaccine vesicles exuded from the vesicles, and their subsequent course was identical with that of vaccinal lesions of a severe and prolonged attack of generalised vaccinia. The farmer himself had milked the cows and washed their udders. He had not been vaccinated in the ordinary way since childhood.

On April 16th the farmer's son, aged 26 years, who had attended to the cows affected with sore teats and had washed their udders, noticed that both his hands were uncomfortable and on the 17th and 18th papules developed on the palmar and dorsal surfaces, the right hand being considerably more affected than the left. The papules were accompanied by severe local œdema and redness of the surrounding skin. Lymphangitis was set up in the right arm and considerable enlargement of the axillary lymphatic glands, while the temperature of the patient rose to 104° F. on two evenings. The papules became vesicular, showing the distinctive appearance of the vesicles in a case of generalised vaccinia, and their subsequent course was typical of that condition. The patient had not been vaccinated since infancy. On May 17th he allowed me to vaccinate him on his left arm. No vesicle or even papule resulted. The vaccine used was the calf vaccine series which was being issued at the time from these laboratories for human vaccination. The results obtained by medical men with this series are shown by the schedules which have come to hand to be : case success 100 per cent. and insertion success 99·7 per cent. No failures have been reported on any schedule. It may be assumed therefore that the patient on the 17th was immune to vaccination.

On May 1st a farm labourer, aged 39 years, who had been engaged in milking the cows, developed two papules on his right hand. This case followed the same course as the two preceding cases, except that the disease was very much milder. This man had not been previously vaccinated since infancy.

On May 8th another farm labourer, aged 32 years, developed a papule on his right hand. His case was parallel with the one last mentioned. He had not been vaccinated since infancy.

It has been ascertained lately that while the farmer and his son were ill three other cow-milkers said that they had sore fingers on the right hand. Apparently no vesicular stage occurred in these cases, but as their hands were not examined at the time little or no value can be attached to their condition. All the foregoing persons are right-handed.

Only one milker, an old man, says that he has had nothing the matter with his hands.

Bacteriology.—Aerobic and anaerobic cultivations showed the presence of staphylococcus aureus and albus only in the pathological material available—namely, the exudate from the vesicles of the farmer's son's hand. These micro-organisms, as is well-known, are those generally obtained from the vaccine lymph of humans and of animals.

Animal experiments.—For these also the only available pathological material was fluid exudate from the vesicles of the farmer's son's hand. Two guinea-pigs inoculated with this fluid developed typical vaccine vesicles three and four days later at the site of inoculation. Two weeks later these guinea-pigs were inoculated with highly potent calf vaccine and no specific reaction was noticed as a result. A calf inoculated with the fluid from the farmer's son's hand developed typical vaccine vesicles. It was not practicable to vaccinate this animal subsequently with calf vaccine. The vesicles which appeared as a result of this vaccination were collected and a portion of the resulting emulsion was inoculated on a second bovine. This animal also developed typical vaccine vesicles at the site of inoculation.

It seems evident that the pathological condition which affected the men's hands and the cows' udders was vaccinia. Endeavours have been made to ascertain how the condition first appeared in the cows but so far without success. No persons attending the cows had been vaccinated recently nor had any member of their families. The only other disease noticed among the farm animals was a few cases of foot-rot in sheep ; and, from the experiments which I have made, foot-rot material inoculated on guinea-pigs, calves, and goats, not only sets up no pathological condition resembling vaccinia, but affords no protection against subsequent inoculations with calf vaccine.

The farmer states that his cows have on previous occasions suffered from sore teats, but according to his observations in not the same manner as on the present occasion. There is a possibility, therefore, that the vaccinia may have been endemic on the farm for some time. But the fact that no cow-men have contracted vaccinia previously contradicts such a supposition.

I am greatly indebted to Dr. Wilson, under whose care were all the patients, for his kindness in affording me readily every facility for investigation in his power and for much valuable help ; and to my colleague, Dr. A. T. MacConkey, who during my brief absence from work was notified of the outbreak, for generously handing over the matter to my department on my return to Elstree.

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The Alcoholic Ferment of Yeast-juice. Part III.—*The Function of Phosphates in the Fermentation of Glucose by Yeast-juice.*

By ARTHUR HARDEN and WILLIAM JOHN YOUNG (Biochemical Laboratory of the Lister Institute of Preventive Medicine).

(Communicated by C. J. Martin, F.R.S. Received March 6,—Read April 2, 1908.)

In a previous communication the authors have shown* that when a soluble phosphate is added to a fermenting mixture of glucose and yeast-juice the following phenomena are to be observed: (1) The rate of fermentation is at once greatly increased. (2) This acceleration lasts for a short time and the rate then falls off, and returns approximately to its original value. (3) During this period the extra amount of carbon dioxide evolved and alcohol produced are equivalent to the phosphate added. (4) The phosphate is converted into a form which is not precipitable by magnesia-mixture, and is then probably present as a salt of a hexosephosphoric acid.

(1) *Effect of the Addition of Phosphate on the Total Fermentation.*

The addition of phosphate, however, does not simply produce this initial decomposition of an equivalent of glucose, but also, as a rule, a greater total fermentation, after allowance has been made for the amount decomposed during the initial period.

This is clearly shown by the results embodied in the following table.

In each experiment two or more portions of 25 c.c. of yeast-juice were taken, a solution of glucose alone, or one of glucose and phosphate, added, and the total volume made to 50 c.c. The solution of phosphate employed had a concentration of about 0·3 molar, and the concentration of glucose was 20 grammes per 100 c.c. in experiment 8, and 10 grammes per 100 c.c. in all the others. The fermentation was carried out at 25° in presence of toluene until the evolution of gas ceased. The numbers in the last column show the increase in the total fermentation produced during the period subsequent to the initial acceleration.

It will be seen that the increase which occurs after the initial period varies from about 10 per cent. of the original fermentation to as much as 150 per cent.

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* 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 405.

Experiment.	CO_2 evolved without phosphate.	Cubic centimetres of phosphate solution added.	CO_2 evolved in presence of phosphate.	Increase due to phosphate.	CO_2 equivalent to phosphate.	Increase after initial period.
	grammes.	c.c.	grammes.	gramme.	gramme.	gramme.
1	0·484	10	0·717	0·233	0·132	0·101
2	1·280	5	1·584	0·304	0·066	0·238
3*a*	0·422	10	0·634	0·212	0·132	0·080
3*b*	0·422	20	0·748	0·326	0·264	0·062
4	0·440	10	1·258	0·818	0·132	0·686
5	0·405	5	0·515	0·110	0·070	0·040
6	0·603	5	0·735	0·132	0·066	0·066
7	0·438	5	0·593	0·155	0·057	0·098
8	1·016	15	1·632	0·616	0·198	0·418
	c.c.		c.c.	c.c.	c.c.	c.c.
9	369	10	629	260	63	197
10	337	10	569	232	56	176

(2) *Recurrence of Phosphate.*

The reason for this increase in the amount of sugar decomposed in the long period following the short initial period of acceleration appears to be that the phosphorus compound first formed, which is a hexosephosphate of the formula $C_6H_{10}O_4(PO_4R_2)_2$,* is slowly hydrolysed, probably by an enzyme, with the production of a phosphate and a hexose. The phosphate is thus slowly regenerated and then again undergoes the reaction, causing an increased fermentation in the same manner as when it was originally added.

This recurrence of phosphate is clearly shown by the following experiment. A known amount of phosphate was added to yeast-juice containing glucose, and the mixture incubated at 25° with toluene. At the close of the initial period a sample was removed, boiled and filtered, and the free and total phosphate present in it estimated, and this process was repeated at stated times. The results obtained are given below, the amounts of phosphate being expressed in grammes of $Mg_2P_2O_7$ per 10 c.c.

Experiment 11.—215 c.c. of yeast-juice + 20 grammes of glucose were made to 375 c.c. with a solution of potassium phosphate, the amount of the latter being equivalent to 0·133 gramme $Mg_2P_2O_7$ per 10 c.c. of the resulting liquid.

The slight increase in the total phosphate present is due to a corresponding degree of evaporation during the experiment. It will be seen that the free phosphate per 10 c.c. gradually increases from 0·021 to 0·226, so that 0·205 gramme is regenerated. Since the total phosphorus, expressed as phosphate in

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* Young, 'Chem. Soc. Proc.,' 1907, vol. 65.

Time in hours.	Free phosphate as $Mg_2P_2O_7$ per 10 c.c.	Total phosphate as $Mg_2P_2O_7$ per 10 c.c.
	gramme.	gramme.
5·5	0·021	0·266
18·0	0·093	0·269
66·0	0. 133	
138·0	0·175	
426·0	0·226	0·273

the original juice, was 0·266 − 0·133 = 0·133 gramme, it follows that at least 0·205 − 0·133 = 0·072 gramme of this has been derived from the hexose-phosphate produced during the initial period from the added phosphate.

Even in the absence of added phosphate a gradual production of free phosphate occurs when yeast-juice is incubated at 25°. In the absence of glucose, when the fermentation is very small, the increase in the amount of free phosphate is comparatively rapid, whereas, in the presence of glucose, the fermentation lasts for a considerable period, and the appearance of free phosphate is delayed, since it continually enters into fresh combination as rapidly as it is formed.

Examples of this are the following. The various materials were digested at 25° with toluene for the time shown, and were then boiled and filtered and the free phosphate estimated.

Experiment.	Material digested.	Time of digestion.	Free phosphate as $Mg_2P_2O_7$ per 25 c.c.
		days.	gramme.
12	(*a*) Yeast-juice alone	0	0·128
	(*b*) ,, ,, ...	2	0·284
	(*c*) ,, ,, ...	10	0·283
	(*d*) Yeast-juice + glucose	2	0·123
	(*e*) ,, ,, ...	10	0·255
13	(*a*) Yeast-juice alone	0	0·024
	(*b*) ,, ,, ...	1	0·053
	(*c*) ,, ,, ...	2	0·063
	(*d*) ,, ,, ...	4	0·069
	(*e*) Yeast-juice + glucose	1	0·011
	(*f*) ,, ,, ...	2	0·025
	(*g*) ,, ,, ...	4	0·051
		hours.	
14	(*a*) Yeast-juice + glucose + phosphate (0·276 gramme per 25 c.c.)	1·25	0·063
	(*b*) ,, ,, ,,	46·0	0·138
	(*c*) ,, ,, ,,	96·0	0·387
	(*d*) ,, ,, ,,	168·0	0·453
	(*e*) ,, ,, ,,	240·0	0·489
	(*f*) ,, ,, ,,	336·0	0·501
	(*g*) ,, ,, ,,	456·0	0·523

In this last experiment (No. 14) sample (*a*) was taken at the close of the initial period and sample (*b*) immediately after the cessation of fermentation, these points being determined by observation of another sample of the yeast-juice.

It is to be noted that during the fermentation only a small increase occurs in the amount of free phosphate (0·075 gramme), while after the cessation of fermentation the increase amounts to about three times as much (0·249 gramme) in approximately an equal time. The total phosphate of the original juice was 0·350 gramme per 25 c.c. and the amount added was 0·276 gramme. Since only 0·104 gramme of phosphate remains combined at the close of the experiment, it follows that at least $0·276 - 0·104 = 0·172$ gramme has been regenerated from the hexosephosphate.

The recurrence of phosphate in these cases appears to be due to the action of an enzyme—whether a special enzyme or one of those already known to occur in yeast-juice has not yet been determined.

After yeast-juice containing the sodium salt of hexosephosphoric acid has been boiled the amount of free phosphate remains practically unaltered when the liquid is incubated at 37°, as is shown by the following experiment.

Experiment 15.—To yeast-juice containing glucose and an amount of free phosphate corresponding to 0·184 gramme of $Mg_2P_2O_7$ per 25 c.c. was added sodium phosphate equivalent to 0·285 gramme of $Mg_2P_2O_7$ and the mixture incubated at 37° in presence of toluene. A sample was taken at the close of the initial period, and as soon as fermentation had almost ceased the whole was boiled, the free phosphate estimated and the remainder of the boiled liquid preserved at 37°, samples being taken at intervals.

At the close of the initial period, 25 c.c. yielded only 0·04 gramme of $Mg_2P_2O_7$, so that practically the whole of the added phosphate must have been converted into a salt of hexosephosphoric acid. At the cessation of fermentation the same volume yielded 0·131 gramme of $Mg_2P_2O_7$, so that at least 0·15 gramme was still present as a hexosephosphate.

After incubation of the boiled liquid at 37° for an additional 144 hours, the amount of free phosphate had only increased to 0·138 gramme, so that the action practically ceased when the liquid was boiled.

(3) *Nature of the Chemical Change which occurs in the Fermentation of Glucose by Yeast-juice.*

The cycle of changes which is undergone by a phosphate in the presence of yeast-juice and glucose appears from the foregoing to be as follows:—

(1) $2C_6H_{12}O_6 + 2R_2HPO_4 = 2CO_2 + 2C_2H_6O + C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O.$

(2) $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2R_2HPO_4.$

The first of these equations does not include the fermenting complex, without which, however, the change does not occur, and it is probable that both the glucose and the phosphate form an intermediate association with this complex, which then breaks down, giving rise to the substances on the right-hand side of the equation, and at the same time regenerating the fermenting complex.

Since free phosphate and a hexosephosphate are invariably present in the yeast-juice prepared by grinding yeast, it follows that at all events some portion of the fermentation is always due to the foregoing reactions. During the initial period of rapid fermentation, as long as free phosphate is still present, the greater part of the change is certainly due to this reaction, whilst in the succeeding period of slower fermentation the constant production of free phosphate by the enzymatic hydrolysis of the hexosephosphate already formed, or by the action of proteoclastic enzymes on phosphoproteins, renders it equally certain that some portion of this greatly diminished fermentation must also be ascribed to the same reaction.

The question at once arises whether it is not possible that the whole of the fermentation is due to this reaction. So far, no fact has been encountered which is inconsistent with this view. In previous communications* the authors have shown that at least two substances are concerned in the production of carbon dioxide and alcohol from glucose: the ferment, which is thermolabile, and the coferment, which is thermostable; and, further, that phosphates are incapable of producing any fermentation when they are added to a mixture of the ferment and glucose in the absence of the coferment.

If the theory suggested above be found to be correct, it will be necessary to complete this statement. Two possibilities present themselves. Either a third substance, a soluble phosphate, is also necessary, and in the presence of the fermenting complex, made up of ferment and coferment, undergoes the reaction under discussion; or the coferment may itself be a complex substance containing a group of unknown composition, united with the phosphoric acid group. The latter would then be passed on to the glucose during fermentation, and a new group taken up from the phosphate, a continuous conversion of phosphate into hexosephosphate being thus effected.

If this cycle of changes correctly represents the reaction which occurs, it follows that the rate of fermentation after the initial period of acceleration depends, in the first instance, on the rate at which phosphate is liberated according to equation (2).

The most satisfactory method of testing the accuracy of this view would be to free both ferment and coferment from phosphate and materials capable of yielding phosphates under the influence of the enzymes which are present, and then to ascertain whether the mixture of these purified materials would ferment glucose; and, further, if they did not, whether the addition of a phosphate would bring about fermentation. Many attempts have been made to realise these conditions, but hitherto without success. It is obvious that if the coferment be a hydrolysable derivative of phosphoric acid, as suggested above, success in this direction cannot be anticipated.

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* 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 405; B, vol. 78, 1906, p. 369.

Some idea of the extent to which alcoholic fermentation is due to this recurrence of phosphate could also be gained by ascertaining the amount of free phosphate produced from hexosephosphate in yeast-juice in a given time in the absence of glucose, and comparing this with the amount of carbon dioxide evolved in the presence of glucose under otherwise identical conditions.

In practice, however, two difficulties present themselves. In the first place, in the absence of fermentable sugar, or when the concentration of this is very low, free phosphate accumulates, and the rate of hydrolysis of the hexosephosphate is thus diminished; whereas in the presence of glucose the concentration of phosphate remains constant at a low value during fermentation, and the rate of hydrolysis of hexosephosphate accordingly remains at its maximum for a considerable period.

This inhibitory effect of phosphate on the hydrolysis of hexosephosphate in the absence of all fermentation is shown in the following experiment, which also indicates the extent to which this hydrolysis occurs under these conditions.

Experiment 16.—In order to avoid all fermentation, the inactive residue obtained by filtering yeast-juice through a Martin gelatin-filter was employed, and equal weights of this were incubated for 20 hours with: (1) water; (2) a solution of sodium hexosephosphate free from glucose and free phosphate; (3) a solution of sodium hexosephosphate + an equivalent amount of sodium phosphate. A solution of hexosephosphate was also incubated alone, the conditions of concentration and alkalinity being identical in all four solutions. At the expiration of 20 hours, the solutions were all boiled, and the free phosphate in the filtrates estimated. In the following table the numbers represent the weight of $Mg_2P_2O_7$ found.

The numbers in the last column are obtained by adding together the amount of phosphate produced separately by the incubation of the hexosephosphate and the residue, and subtracting their sum from the free phosphate produced in the other two solutions.

It appears from this that in this particular instance the enzymatic

Solution.	Amount of free phosphate present.		Amount of hexosephosphate hydrolysed by enzyme.
	Before incubation.	After incubation.	
1. Hexosephosphate equivalent to 0·1626 gramme $Mg_2P_2O_7$	0·0	0·0089	
2. 0·75 gramme residue + water ……	0·0	0·0166	
3. 0·75 gramme residue + hexosephosphate equivalent to 0·1626 gramme $Mg_2P_2O_7$	0·0	0·1068	0·0813
4. 0·75 gramme residue + hexosephosphate as above, + sodium phosphate equivalent to 0·1626 gramme $Mg_2P_2O_7$	0·1626	0·1884	0·0003

decomposition of the hexosephosphate is almost completely arrested by the presence of an equivalent of free phosphate.

The amount of phosphate actually produced in solution 3 from all sources is 0·1068 gramme $Mg_2P_2O_7$, which corresponds to an evolution of about 22 c.c. of carbon dioxide, and this in spite of the fact that the phosphate has been allowed to accumulate. The amount which would be evolved in presence of glucose and coferment would naturally exceed this, owing to the continued reconversion of the phosphate into hexosephosphate.

This experiment also shows that the ferment which brings about the hydrolysis of the hexosephosphate is present in the residue obtained by filtering yeast-juice through a Martin-filter, and does not require a dialysable coferment.

The second difficulty arises from the fact that when yeast-juice or a mixture of ferment and coferment is employed, a certain amount of fermentation always occurs, even in the absence of added sugar. This is due to sugar formed in the liquid, in part by the hydrolysis of glycogen and dextrins and in part by the hydrolysis of the hexosephosphate itself, which yields a fermentable sugar as one of its products. The practical result is that if an actual comparison be instituted between the production of phosphate in the absence of added glucose and the evolution of carbon dioxide in the presence of glucose, it will be necessary to take the sum of the carbon dioxide actually evolved and the carbon dioxide equivalent of the phosphate produced in the absence of added glucose, and this will always be found to be less than the volume of carbon dioxide observed in the presence of glucose. The result of such a comparison is shown in the two following experiments.

A mixture was made of yeast-juice with a solution containing a suitable amount of sodium phosphate and just sufficient glucose to bring about the conversion of the greater portion of the phosphate into hexosephosphate, this being found by experiment to be rather more than an equivalent of glucose. The mixture was incubated until this conversion had been accomplished and the rate of fermentation had become steady. A sample was then taken and boiled and the free phosphate estimated in the filtrate. The remainder was incubated for a further period, the evolution of gas being noted, and a second sample was then taken.

A parallel experiment was carried out with yeast-juice containing the same amount of sodium phosphate in presence of 10 grammes of glucose per 100 c.c., and the evolution of gas during the same period was observed. The solutions used and the results obtained were as follows:—

Experiment 17.—(1) 100 c.c. yeast-juice + 30 c.c. of a solution containing 1·3 grammes sodium phosphate and 2·62 grammes glucose. (2) 100 c.c. yeast-juice + 30 c.c. of a solution containing 1·3 grammes sodium phosphate and 13 grammes glucose.

Experiment 18.—(1) 100 c.c. yeast-juice + 40 c.c. of a solution containing 1·7 grammes sodium phosphate and 3·16 grammes glucose. (2) 100 c.c. yeast-juice + 40 c.c. of a solution containing 1·7 grammes sodium phosphate and 14 grammes glucose.

Experiment.	Solution 1 (low concentration of glucose).						Solution 2 (excess of glucose).
	Original phosphate.	Final phosphate.	Phosphate produced.	CO_2 equivalent.	CO_2 evolved.	Total CO_2 equivalent.	CO_2 evolved.
				c.c.	c.c.		
17	0·0559	0·1052	0·0493	10·7	39·8	50·5	93·9
18	0·1036	0·1701	0·0665	14·6	27·7	42·3	69·2

These experiments show that although the sum of the carbon dioxide evolved and that equivalent to the phosphate produced in absence of glucose is invariably less than the amount of carbon dioxide evolved in the presence of glucose, yet the difference is no greater than might be expected from a knowledge of the prevailing conditions, and is quite consistent with the view that the whole of the fermentation proceeds according to the equation proposed.

(4) *Influence of Concentration of Phosphate on the Course of the Fermentation.*

When a phosphate is added to a fermenting mixture of glucose and yeast-juice, the effect varies both with the concentration of the phosphate and with the particular specimen of yeast-juice employed. With low concentrations of phosphate the acceleration produced is so transient that no accurate measurements of rate can be made. As soon as the amount of phosphate added is sufficiently large, it is found that the rate of evolution of carbon dioxide suddenly increases from 5 to 10 times, and then rapidly falls approximately to its original value.

As the concentration of phosphate is still further increased, it is first observed that the maximum velocity, which is still attained immediately on the addition of the phosphate, is maintained for a certain period before the fall commences, and then, as the increase in concentration of phosphate proceeds, that the maximum is only gradually attained after the addition, the period required for this increasing with the concentration of the phosphate. Moreover, with these higher concentrations the maximum rate attained is less than that reached with lower concentrations, and, further, the rate falls off more slowly. The concentration of phosphate which produces the highest rate, which may be termed the optimum concentration, varies very considerably with different specimens of yeast-juice.

All these points are illustrated by the accompanying tables and curves.

Experiment 19.—The following solutions were employed:—

(1) 25 c.c. yeast-juice + 15 c.c. sodium bicarbonate solution.

(2) 25 c.c. yeast-juice + 10 c.c. of potassium phosphate solution (0·3 molar) + 5 c.c. sodium bicarbonate solution.

(3) 25 c.c. yeast-juice + 15 c.c. potassium phosphate solution. All the solutions contained 4 grammes of glucose, and the experiment was carried out at 25° in the presence of toluene. The solution of bicarbonate added contained an amount of this salt equal to that formed by the action of carbon dioxide on an equal volume of the solution of potassium phosphate employed.

Time after addition in minutes.	Carbon dioxide evolved in preceding 5 minutes with n cubic centimetres of 0·3 molar potassium phosphate added.		
	n = 0 c.c.	n = 10 c.c.	n = 15 c.c.
5	4·0	11·1	7·7
10	3·2	16·0	9·7
15	4·2	20·2	12·1
20	3·6	**22·4**	16·1
25	4·3	17·4	18·4
30	3·6	6·6	19·4
35	4·3	4·6	**20·4**
40	3·2	4·7	16·7
45	—	4·5	12·7
50	—	4·2	6·0
55	—	4·1	4·0

Experiment 20.—

(1) 25 c.c yeast-juice + 5 c.c. phosphate + 15 c.c. bicarbonate.
(2) 25 c.c. „ + 10 c.c. „ + 10 c.c. „
(3) 25 c.c. „ + 15 c.c. „ + 5 c.c. „
(4) 25 c.c. „ + 20 c.c. „ + 0 c.c. „

The concentration of glucose was 4·5 grammes per 45 c.c., and the experiment was carried out at 25° in presence of toluene.

Time after addition in minutes.	Carbon dioxide evolved in preceding 5 minutes with *n* cubic centimetres of 0·3 molar phosphate added.			
	n = 5 c.c.	*n* = 10 c.c.	*n* = 15 c.c.	*n* = 20 c.c.
5	7·1	2·7	1·6	1·6
10	12·7	6·2	2·4	1·7
15	**16·3**	8·1	3·0	2·0
20	7·7	9·6	3·3	2·0
25	1·9	12·4	4·0	2·3
30	1·8	14·5	5·1	2·6
35	—	**16·8**	6·0	2·7
40	—	15·0	6·5	3·1
45	—	2·9	7·3	3·5
50	—	1·7	8·5	3·8
55	—	—	**8·7**	4·4
60	—	—	8·5	4·9
65	—	—	8·6	5·6
70	—	—	8·1	5·7
75	—	—	7·6	5·6
80	—	—	7·0	**6·0**
85	—	—	6·0	6·0
90	—	—	—	6·0
95	—	—	—	5·5
100	—	—	—	5·4
105	—	—	—	5·0

Curves A, B, C, and D (fig. 1) show the rate of evolution per five minutes for the four solutions in experiment 20. The time of addition is taken as zero, the rate before addition being constant, as shown in the curves.

It will be observed that in experiment 19 practically the same maximum is attained with 10 and 15 c.c. of phosphate, whereas, in experiment 20, 5 and 10 c.c. give the same maximum, whilst 15 c.c. produce a much lower maximum, and 20 c.c. a still lower one, the rate at which the velocity diminishes after the attainment of the maximum being correspondingly slow in these last two cases. By calculating the amount of phosphate which has disappeared as such from the amount of carbon dioxide evolved, it is found that the maximum does not occur at the same concentration of free phosphate in each case.

These results suggest that the phosphate is capable of forming two or

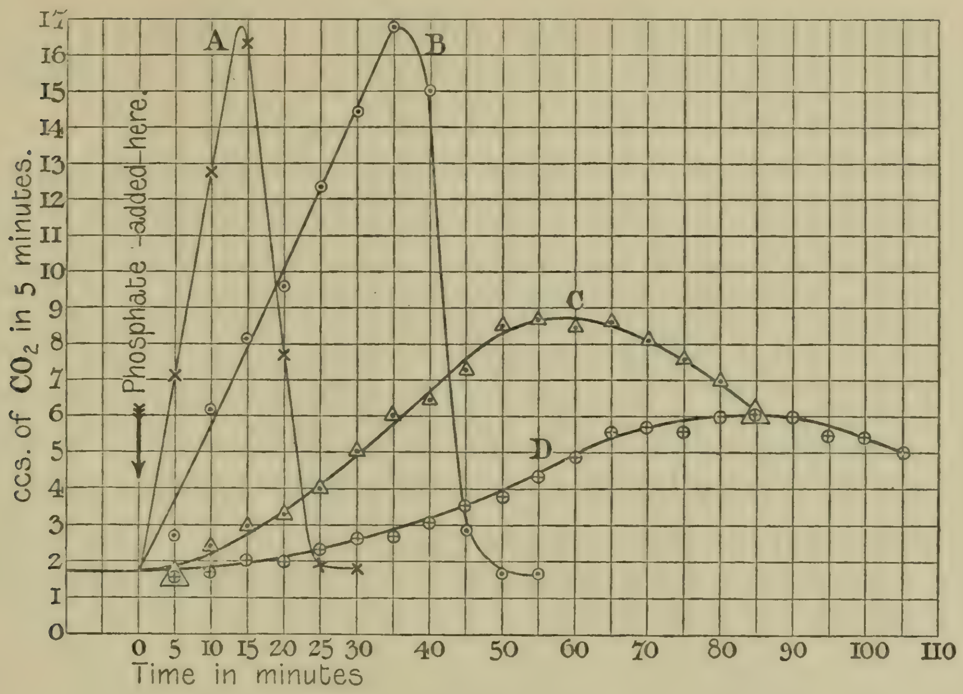

Fig. 1.

more different unstable associations with the fermenting complex. One of these, formed with low concentrations of the phosphate, has the composition most favourable for the decomposition of sugar, whilst the others, formed with high concentrations of phosphate, contain more of the latter, probably associated in such a way with the fermenting complex as to render the latter partially or wholly incapable of effecting the decomposition of the sugar molecule. As the fermentation proceeds slowly in the presence of excess of phosphate, the concentration of the latter is reduced by conversion into hexosephosphate, and a redistribution of phosphate occurs, resulting in the gradual change of the less active into the more active association of phosphate with fermenting complex, and a consequent rise in the rate of fermentation.

In those cases in which the maximum rate corresponding to the optimum concentration of phosphate is never attained, some secondary cause may be supposed to intervene, such as a permanent change in a portion of the fermenting complex, accumulation of the products of the reaction, etc. Experiments on this point are being carried out by varying independently the concentration of the ferment, the coferment, the phosphate, and the hexosephosphate.

In agreement with these conclusions it is found that a high rate of evolution of carbon dioxide can be maintained for a considerable period by the gradual addition of phosphate in such a way that the concentration of free phosphate remains approximately at the optimum value. This may be effected by adding in every interval of five minutes the amount of phosphate equivalent to the carbon dioxide evolved in excess of the normal rate of fermentation of the yeast-juice during the same interval of time.

Experiment 21.—25 c.c. of yeast-juice + 2·5 grammes glucose were incubated at 25° in the presence of toluene until the rate became constant. A solution of potassium phosphate (2 mol. K_2HPO_4 and 1 mol. of KH_2PO_4) of 0·3 molar concentration was then gradually added from a graduated pipette provided with a tap and passing through the cork of the fermentation flask. Column 2 shows the c.c. of this added in each period of five minutes, and column 3 the evolution of carbon dioxide during each period of five minutes. The normal rate of the yeast-juice, in the absence of added phosphate, was 3 c.c. per five minutes. It will be seen that an average rate of about 15 was maintained for an hour and a quarter, 32 c.c. of the phosphate solution being added in all.

1. Time in minutes.	2. Cubic centimetres of phosphate solution added in each 5 minutes.	3. Evolution of carbon dioxide in each 5 minutes.
	c.c.	
5	5·0	3·1
10	1·5	15·4
15	2·0	16·2
20	1·0	20·2
25	2·5	17·2
30	1·5	12·7
35	2·0	15·7
40	0·0	17·4
45	0·0	9·6
50	3·0	8·4
55	3·0	15·4
60	3·0	20·2
65	3·0	17·2
70	3·0	14·8
75	1·5	14·9

Further experiments on this subject are in progress, particularly with respect to the relations between phosphate and coferment, and the bearing of these phenomena on the fermentation of sugars by living yeast.

Summary.

1. The addition of a phosphate to a fermenting mixture of glucose and yeast-juice not only produces a temporary acceleration in the rate of fermentation, but, in addition to this, an increased total fermentation.

2. This last effect is due to the fact that the hexosephosphate formed during

the period of temporary acceleration is continually hydrolysed by an enzyme, with production of free phosphate, which again enters into reaction and thus brings about an increased fermentation.

3. It appears probable that the presence of phosphate is essential for the alcoholic fermentation of glucose by yeast-juice, the reaction which occurs being the following :—

(1) $2C_6H_{12}O_6 + 2R_2HPO_4 = 2CO_2 + 2C_2H_6O + C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O$.

This reaction is only realised in the presence of the ferment and coferment discussed in previous communications, phosphate alone being unable, in the absence of coferment, to bring about fermentation in a mixture of ferment and glucose.

The hexosephosphate thus formed is then hydrolysed :

(2) $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2R_2HPO_4$.

The rate at which this second reaction occurs determines the rate of fermentation observed when glucose is fermented by yeast-juice.

4. An optimum concentration of phosphate exists which produces a maximum initial rate of fermentation. Increase of concentration beyond this optimum diminishes the rate of fermentation.

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THE EFFECTS OF MUSCULAR WORK UPON THE EXCRETION OF ENDOGENOUS PURINES.

By E. L. KENNAWAY, *British Medical Association Research Scholar.*

(*From the Lister Institute of Preventive Medicine.*)

CONTENTS.

		PAGE
1.	Introduction	1
2.	The effect of repetition of work	3
3.	The simultaneous effects of muscular work and of salicylic acid	11
4.	The excretion of purine bases during muscular work	14
5.	The diurnal variations in the excretion of endogenous purine bases	19
6.	The relations between the amounts of urine, and of uric acid and purine bases	19
7.	The amount of the endogenous purine output	20
8.	Methods of estimation	23
	Summary	24
	Protocols	26

THIS subject has been investigated previously in the course of Leathes' work upon endogenous uric acid, and the results obtained have been published in a paper by him and others [(1)]. The effects of a single period of unaccustomed exertion were shown in this paper by several examples. Briefly, these effects may be said to be (1) during the work, a great diminution in the output of uric acid, and increase in that of the purine bases, (2) at or about the close of the period of work, a great and rapid rise in the excretion of uric acid, the output of purine bases remaining for a time at a high level, (3) subsequently, the persistence of a large excretion of uric acid for as long as 48 hours; during this period, indications of a diurnal wave of the usual character [(2)] may be dectected. This series of changes can be followed in Fig. 1.

The investigations described in the present paper were undertaken to elucidate the conditions underlying some of these changes.

In accordance with prevalent ideas as to the metabolism of uric acid, the large output of this substance which follows muscular work might be attributed to the flushing-out of an amount of it which would not otherwise have reached the urine, although present in the tissues whether exercise be or be not taken. On the other hand, greater activity of the processes leading to the formation of uric acid would explain the increased output; this explanation was put forward in the paper referred to above (1), and some of the experiments described in the present paper were designed to test its accuracy. With this object, attention was directed at Dr Leathes' suggestion to the simultaneous effects of exercise and of salicylic acid upon the excretion of uric acid. The latter effect had been observed in the same person on a former occasion (1). The results then obtained suggest that this action consists to a large extent in the elimination of uric acid actually present as such in the tissues, and is therefore of the same nature as the action assigned to muscular work by the first of the two theories mentioned above, but entirely different from that required by the second theory. It appeared then, that if the performance of muscular work were combined with the ingestion of salicylic acid, evidence would be obtained which would show whether these two agents increased the output of uric acid by the same or by different modes of action, and would therefore support either the one or the other of the two theories as to the effect of muscular work.

In the second place, experiments were carried out to ascertain whether the changes in the excretion of purines observed during and after a period of unaccustomed exercise would be produced on subsequent occasions when the same amount of work was performed by the same muscles, or whether it were necessary that the exercise should employ muscles which had not for some time been used in this way or to this degree. The results obtained will be described before those of the experiment in which salicylic acid was taken. Lastly, a more recent series of observations as to the oxidation of purine compounds during muscular work is included in this paper.

The first series of experiments was made in July 1908, and was carried out upon the system employed previously (1). 230 g. of bread and 750 c.c. of milk were taken at 9 a.m., 2 p.m., and 7 p.m. from June 30th to July 26th. The urine was collected between four times of the day (9 a.m., 2 p.m., 7 p.m. and midnight). On five of the days (July 6th, 12th, 15th, 18th and 20th) exercise was taken from 9.30 a.m. to 11.30 a.m.; the urine passed during this period, and that passed during the next two and a half hours, were examined separately. On these days the first meal was not taken, 750 c.c. of water being taken instead.

The effect of repetition of work.

On four occasions (July 6th, 12th, 15th, 18th) the same amount of work was performed (10 lb. in each hand lifted from the floor to above the head 850 times in the course of two hours). The results obtained on July 12th are, owing to the action of the salicylic acid taken on that day, not directly comparable with those obtained on the other three occasions; it will be shown, however, that this experiment yields figures which conform to a certain progressive change to be observed in this series of four days of work.

On the next day after the first performance of the work, severe stiffness was felt in the muscles of the back and in the hamstrings. After the next occasion (July 12th) no stiffness was felt until the second day following, and it was then only very slight. This great diminution in the stiffness, and its late appearance, when the work had been performed only once before, may have been connected with some action of the salicylic acid. After the third and fourth days of exercise,

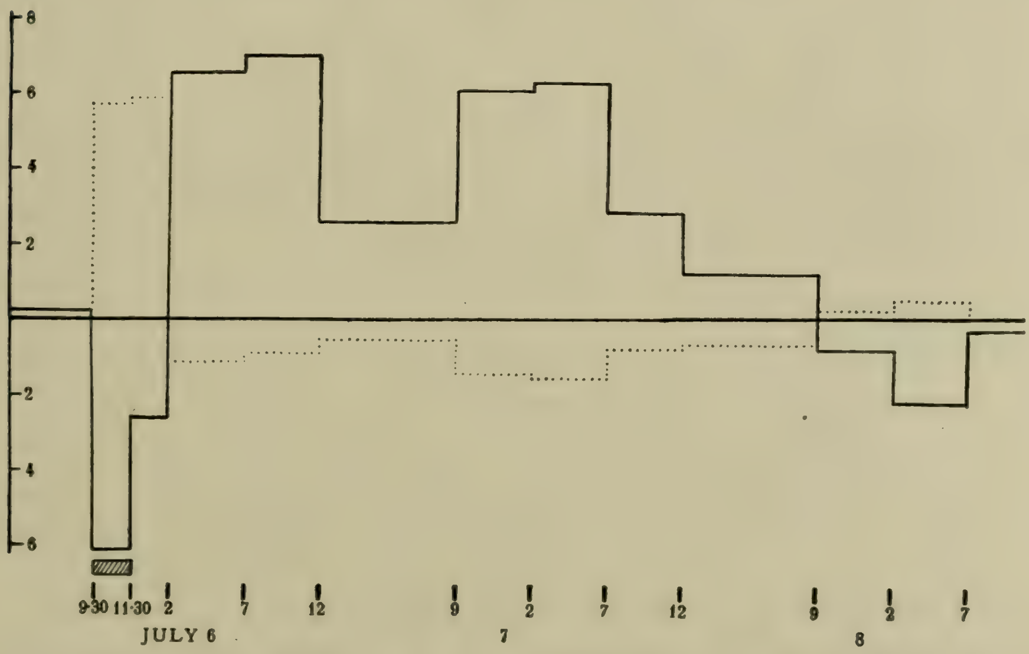

Fig. 1. Exercise (first occasion). July 6th.

Continuous line. Uric Acid Nitrogen. Dotted line. Purine Base Nitrogen.
Abscissæ represent time in hours.
The base line indicates the normal rate of excretion during each of the portions of the day indicated below. Ordinates represent mg. nitrogen per hour above or below the normal rate of the period.
The duration of the exercise is represented thus ▨.

practically no stiffness was present, and the work on these days was performed with hardly any sense of effort; one must conclude, therefore, that the muscles employed were by this time in a well trained condition.

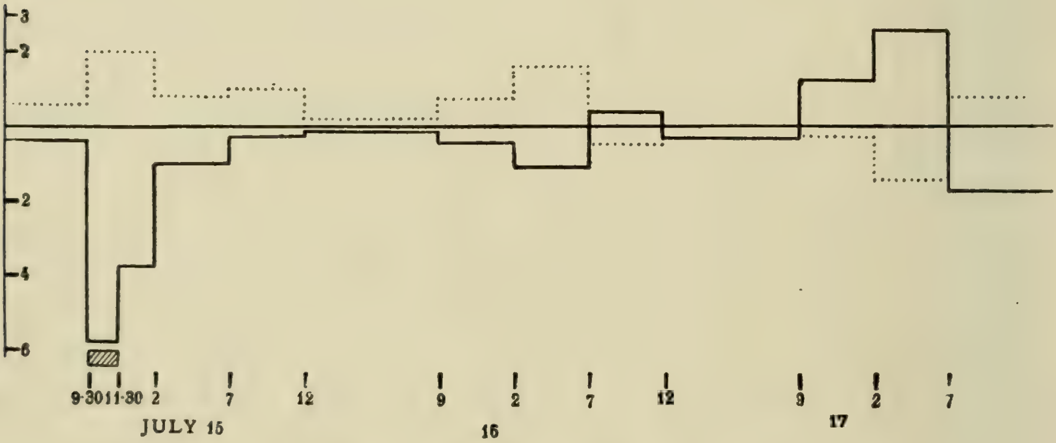

Fig. 2. Exercise (second repetition). July 15th.

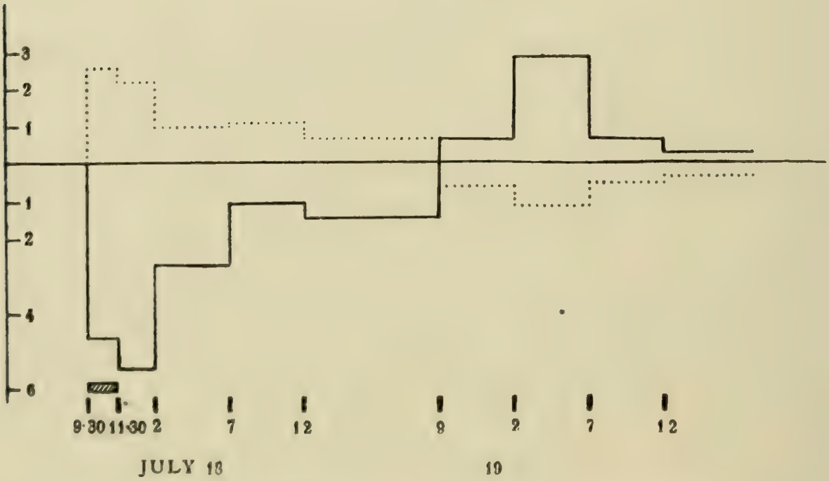

Fig. 3. Exercise (third repetition). July 18th.

The figures show that the disturbance of the normal rates of excretion is considerably greater on the first occasion (Fig. 1), than on the third or fourth (Figs. 2 and 3, see also Table I). With repetition of this form of exercise, the increase of excretion of purine bases during the period of work is lessened. The diminution of the uric acid output during the work is also lessened; while the development of a delay in the rise of uric acid excretion after the work, which rise on the first occasion took place immediately, is especially noticeable.

TABLE I.

U.N. Uric Acid Nitrogen. P.B.N. Purine Base Nitrogen. T.P.N. Total Purine Nitrogen.

	Rates of excretion in mg. per hour					
	Period of work 9.30 a.m. to 11.30 a.m.			11.30 a.m. to 2 p.m.		
	U.N.	P.B.N.	T.P.N.	U.N.	P.B.N.	T.P.N.
July 6th	1·39	7·76	9·15	4·83	7·83	12·66
July 12th, 2 grams salicylic acid taken at 9 a.m.	6·17	7·48	13·65	14·05	2·89	16·94
July 15th	1·59	4·03	5·62	3·73	4·02	7·75
July 18th	2·85	4·59	7·44	2·02	4·25	6·27
July 20th	5·35	6·06	11·41	5·40	5·27	10·67
Averages of above figures (excluding July 12th)	2·80	5·61	8·41	3·99	5·34	9·33
Average rates (9 a.m. to 2 p.m.) for 16 normal days of the July series	7·44	2·02	9·46			
October 20th, without oxygen inhalation	3·07	4·74	7·81	6·36	3·51	9·87
October 28th, oxygen inhalation 9.30 to 2	4·96	4·39	9·35	6·96	2·15	9·11

July 6th, 12th, 15th, 18th, same form of exercise, 20th, new exercise.

In Fig. 4 the amounts of the 24 and 48 hours output of uric acid corresponding to each occasion of exercise, are shown. The time is taken from 9 a.m. preceding the commencement of the work at 9.30. The figures given for July 12th, when salicylic acid was taken, were obtained by subtracting from or adding to the observed outputs the amounts of uric acid above or below the normal average output, which, in an earlier experiment (1), were excreted by the same person in the two periods of 24 hours after taking the same quantity of salicylic acid. On Nov. 25th, 1907, this dose caused in the following 24 hours the appearance in the urine of 115 mg. uric acid nitrogen more than the normal mean output, and in the second 24 hours 51 mg. less than that mean. The actual figures for the 24 hours dating from 9 a.m. on July 12th and 13th respectively, viz. 294 and 101, become after making this correction 179 and 152, which are the figures taken for the construction of Fig. 4. The observed and corrected amounts corresponding to July 12th, together with those which provided the correction, are shown in Fig. 9.

The amounts excreted upon the four consecutive occasions (July 6th, 12th, 15th, 18th), when the same exercise was taken, form a descending series (Fig. 4). It will be seen that the numbers given for the output of the second occasion, when salicylic acid was taken, have positions between the first and third members of the series, which favour the conclusion that, in obtaining these figures, the action of the drug was eliminated with some degree of accuracy. The amounts excreted in the period following the second and third repetitions of the work (July 15th and 18th) are considerably below the normal level, and are thus in marked contrast with the amounts excreted on the first and second occasions. The quantities for the two periods of 48 hours following the second and third repetitions are almost the same, while the output during the first 24 hours is considerably less on the later than on the earlier occasion. The tendency for the phase of high uric acid output which follows exercise to set in later as the exercise is repeated is here

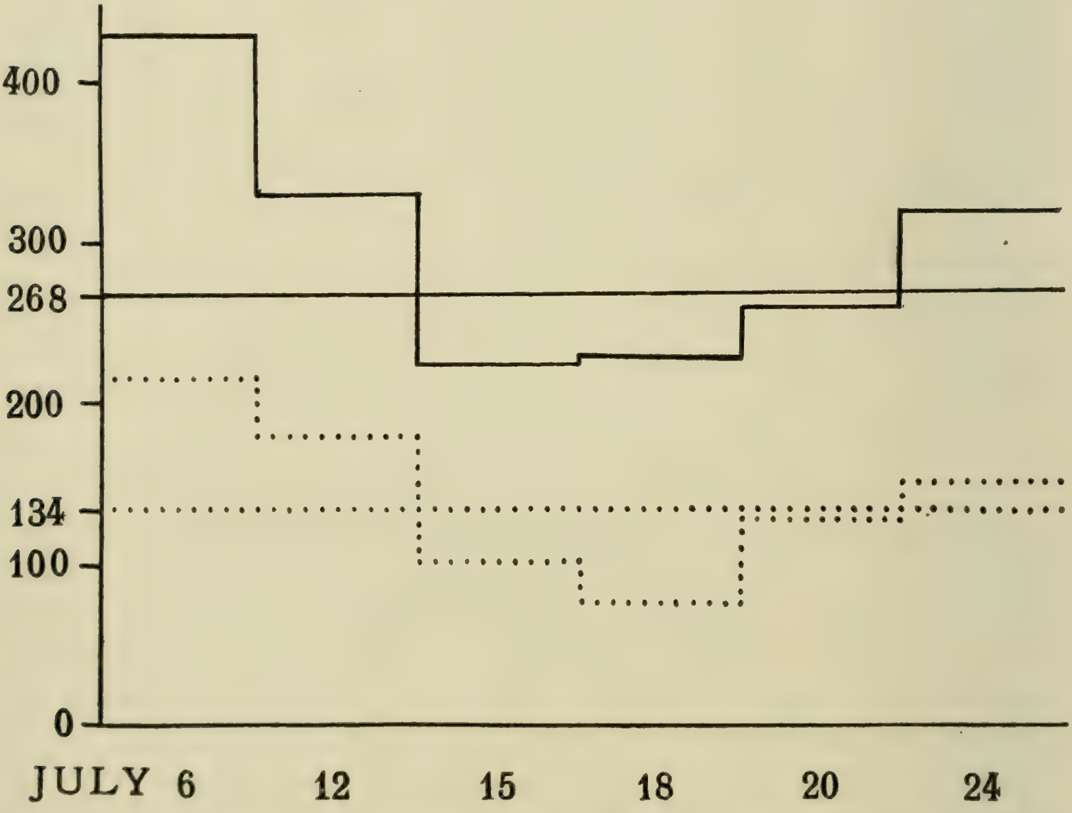

Fig. 4. Output of Uric Acid after Exercise.

Ordinates represent mg. uric acid nitrogen.

Height of dotted lines above base line represents first 24 hours output.

Height of continuous lines above base line represents first and second 24 hours output.

134 mg. Average 24 hours output.
268 mg. Average 48 hours output.

illustrated; allusion was made to this tendency in speaking of the hourly rates of excretion.

The results of this part of the investigation showed, therefore, that the performance of a constant amount of work does not produce constant alterations in the rates of excretion of purines. The changes observed on the first occasion over short periods showed, as the work was repeated, a progressive decrease, and the greatly increased excretion of uric acid present on the days following the first two periods of work was replaced on the two subsequent occasions by an output which is considerably subnormal. One naturally attributed these diminutions in effect on repetition of the same kind and amount of work to training of the particular muscles employed. Accordingly, two forms of exercise of an entirely different kind, employing fresh combinations of muscles, were taken (July 20th and 24th).

On July 20th, the exercise consisted in raising the body 650 times from the lying to the sitting position, a movement brought about chiefly by the muscles of the abdominal wall, which had taken no part in the previous form of exercise. This was varied by raising the body from the prone position until supported on the hands and toes, thus bringing the pectoral muscles into play. In addition, various movements employing the biceps, deltoid, and pectoral muscles were performed with 10 lb. dumb-bells. The duration of the work was the same as on the preceding occasions (9.30 to 11.30 a.m.). During the next two days, severe stiffness was felt in the muscles mentioned above, which had been quite unaffected by the former mode of exercise, after which stiffness was felt in the back and legs only.

The results obtained are shown in Fig. 5. The diminution of uric acid output during the work is even less marked than it was during the last repetition of the previous form of work (Fig. 3). The excretion of purine bases, however, shows an increase during and immediately after the period of work which is considerably greater than that seen on the two preceding occasions (Figs. 2 and 3, see also Table I).

This new form of exercise was employed in order to ascertain whether the activity of a fresh set of untrained muscles would reproduce the changes observed on the first occasion when the previous form of exercise was taken. A comparison of Figs. 1 and 5 shows at once that the great output of uric acid which took place on the first occasion of all was absent after the later period of work of the same duration but of different character. If, however, the results of this new form of work be compared with those yielded by the later repetitions of the previous form (Figs.

2 and 3), it is seen that the new form (1) is accompanied by a greater excretion of purine bases, (2) is followed by an immediate rise of the uric acid output to the normal level, with a subsequent maintenance above this level, no prolonged period of low output being present, and (3) produced during the next 48 hours a much greater output of uric acid (Fig. 4), though this amount is still somewhat below the normal level. The results of the activity of these untrained muscles show, therefore, no continuance of that progressive diminution in effect which was the result of repetition of one mode of exercise. On the contrary, there is a distinct tendency to reproduce the type of change observed on the first occasion of work; the defect in this resemblance is one of degree rather than of kind. A possible explanation of the fact, that this reproduction of the original effects is not more perfect, will be suggested later on (see p. 18).

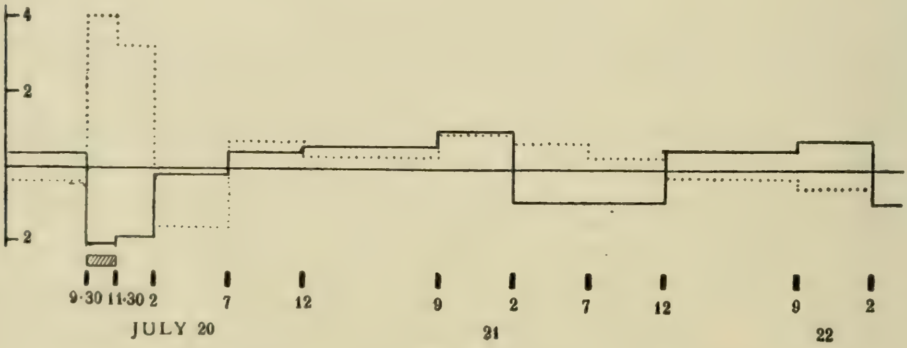

Fig. 5. New exercise (first occasion). July 20th.

Continuous line. Uric Acid Nitrogen. Dotted line. Purine Base Nitrogen.

Abscissæ represent time in hours.

The base line indicates the normal rate of excretion during each of the portions of the day indicated below. Ordinates represent mg. nitrogen per hour above or below the normal rate of the period.

The duration of the exercise is represented thus ▨.

The second new form of exercise was taken on July 24th, and consisted of a walk of 28 miles lasting about nine hours (Fig. 6). No stiffness was felt subsequently. The output of uric acid rose above the normal level during the second half of the walk, and was still above it nearly 48 hours later, when the whole series of analyses was brought to an end. The day of the walk was a very hot one; this condition would tend to lower the output of uric acid, as was shown in the previous paper (1). The amount of purine bases excreted was less than normal both during and after the walk: this lowered output was present throughout the last four days of the series (see protocol I, July 22nd to 26th). This exercise was of course much less severe and more prolonged than that taken on the preceding occasions. It is obvious then, that its results cannot be expected to present any of the details observed on the first occasion. It was thought, however, that resort to this entirely new form of exercise, which involved a longer intermission in the routine of laboratory work, would give a result of some value. This expectation was completely justified, in that the exercise was accompanied and followed by a notable increase in the output of uric acid, the full duration of which was not observed. The output for the 48 hours from the commencement of the walk (321 mg. uric acid nitrogen) is but little lower than that calculated to result from the second performance of the original form of work (332 mg.), the normal output for this period being 268 mg. (Fig. 4). This new form of exercise produced an effect, therefore, which resembled in quantity that seen on the earlier occasions, and such resemblance is all that could be expected.

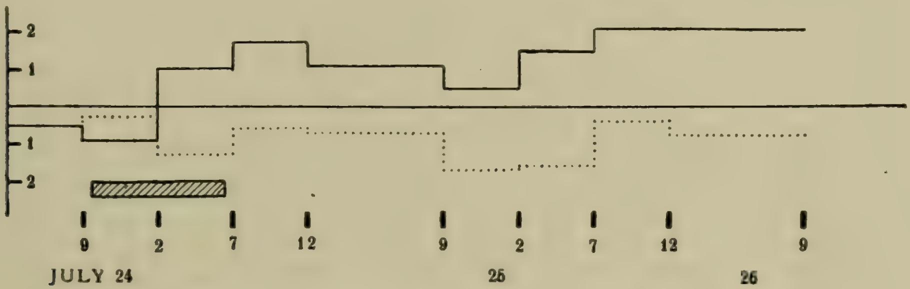

Fig. 6. New exercise (second occasion). Walking. July 24th.

Continuous line. Uric Acid Nitrogen. Dotted line. Purine Base Nitrogen.

Abscissæ represent time in hours.

The base line indicates the normal rate of excretion during each of the portions of the day indicated below. Ordinates represent mg. nitrogen per hour above or below the normal rate of the period.

The duration of the exercise is represented thus ▨.

The result, therefore, of the first of the tests to which the theories referred to at the beginning of this paper were submitted, is favourable to the first of these in so far as the repetition of muscular work of the same kind and amount appears to do away with the effect observed during the 48 hours including and following the work on the first and to a less degree on the second occasion on which it was done; on the third and fourth occasions the net effect on the output of uric acid during this period is a definite diminution (Fig. 4). The fact that the later occasions on which the work was done were not followed by any increased output of uric acid would, in the terms of the first theory in question, appear to signify that the accumulated stock of uric acid was exhausted by the effects of the exercise on the first two occasions. But if that were so neither should work of a different kind be expected, to bring to light evidence of any persisting accumulation. The first of the theories here examined is, that it is not the activity of the muscles themselves which produces uric acid, but other activities accompanying that of the muscles which sweep out uric acid from the body generally. The fact that a new set of muscles is brought into play by a change of work can then make no difference to the output if the accumulated stock is exhausted. As the experiments show, the changed exercises on July 20th gave a result which might be interpreted as due to the exhaustion of the accumulated stock (251 mg. uric acid nitrogen in 48 hours, average 268). But on the other hand the walk on the 24th caused an output in the 48 hour period of more than 50 mg. of uric acid nitrogen above the average. Considered therefore merely in relation to the first of the two theories which the experiments were planned to test, the effect of repetition of muscular activity presents features which are unfavourable, as well as others that may appear to be favourable, to the theory.

It has been suggested (4) that exercise is followed by an increased output of uric acid such as was observed in the experiments recorded in the earlier paper (1), only if the exertions be so severe as to produce actual disintegration of tissue, and that this increase cannot therefore be attributed to heightened activity of the normal processes leading to the formation of uric acid. This objection might be entertained if such results were obtainable only after exercise such as is afforded by the use of heavy dumb-bells. But it cannot be admitted that a walk of 28 miles which is allowed to occupy nine hours and is followed by no stiffness produces anything which can be described as disintegration of tissue, if this term is to denote an effect of the nature of an injury. The subject of these experiments has always noticed that prolonged walking produces no or but very slight stiffness, even when no walks of more than a few miles length have been taken for some months previously. It is evident therefore that the large excretion of uric acid which followed the walk taken on July 24th (Figs. 4 and 6) cannot be ascribed to excessive use of the muscles.

The simultaneous effects of muscular work and of salicylic acid.

The effect of salicylic acid upon the excretion of endogenous uric acid in the person who was the subject of these experiments was observed in the course of an earlier series(1) (Fig. 7). On Nov. 25th, 1907, 4 grams of the acid neutralised with soda were taken at 7.30 p.m. During the first 24 hours following the dose the output of uric acid nitrogen was 115 mg. above the normal amount, and during the second 24 hours 51 mg. below the normal. The net effect in the 48 hours was therefore an increase in the output amounting to 64 mg. Thus the greatly increased output during the 24 hours following a dose of salicylic acid is succeeded by a striking diminution in the output during the second 24 hours (Fig. 9), whereas in the ordinary effect of muscular activity no such diminution is noted (Fig. 1). If the normal output of uric acid is correctly represented as the overflow from a stock of this substance accumulating in the system, then a sudden rise in the output caused by a temporary lowering of the level of the overflow mechanism would be followed by just such a diminished output as a result of the return of the mechanism to its normal working level. The results of this

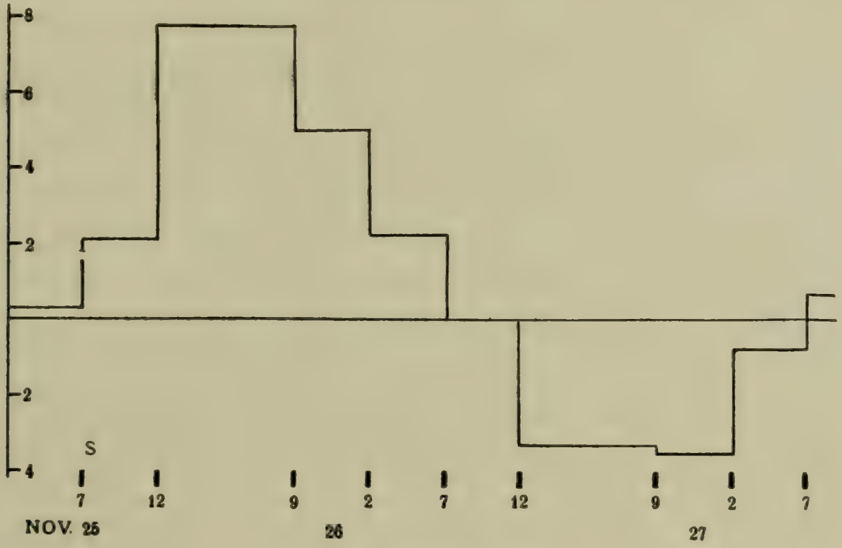

Fig. 7. Salicylic acid.

S. 4 grams salicylic acid taken.

Abscissæ represent time in hours.

The base line indicates the normal rate of excretion of Uric Acid Nitrogen during each of the portions of the day indicated below. Ordinates represent mg. nitrogen per hour above or below the normal rate of the period.

experiment are therefore compatible with the idea that salicylic acid causes the excretion of an amount of uric acid which is present as such in the body, and does not cause any increase in the formation of this substance.

On July 12th, 2 grams of salicylic acid neutralised with soda were taken at 9 a.m. and this dose was repeated at 2 p.m. Between 9.30 and 11.30 a.m., the same work (see p. 3) was performed as on three other days (July 6th, 15th, 18th). During the second hour of work, considerable lassitude was felt, quite distinct from any fatigue due to the exertion. This feeling became extreme during the next few hours, and was noticeable throughout the next day. The effect of the salicylic acid is already evident (Fig. 8) during the period of work, the amount of uric acid excreted during this time being considerably greater than it would be in the absence of the drug's action (cf. Figs. 1 and 2). The output in the 24 hours from the beginning of the work was 294 mg. uric acid nitrogen, and in the second 24 hours 101 mg. (Fig. 9). Supposing that the drug produced the same effect as on the earlier occasion when it was taken, we may subtract from the first of these figures 115, and add to the second 51.

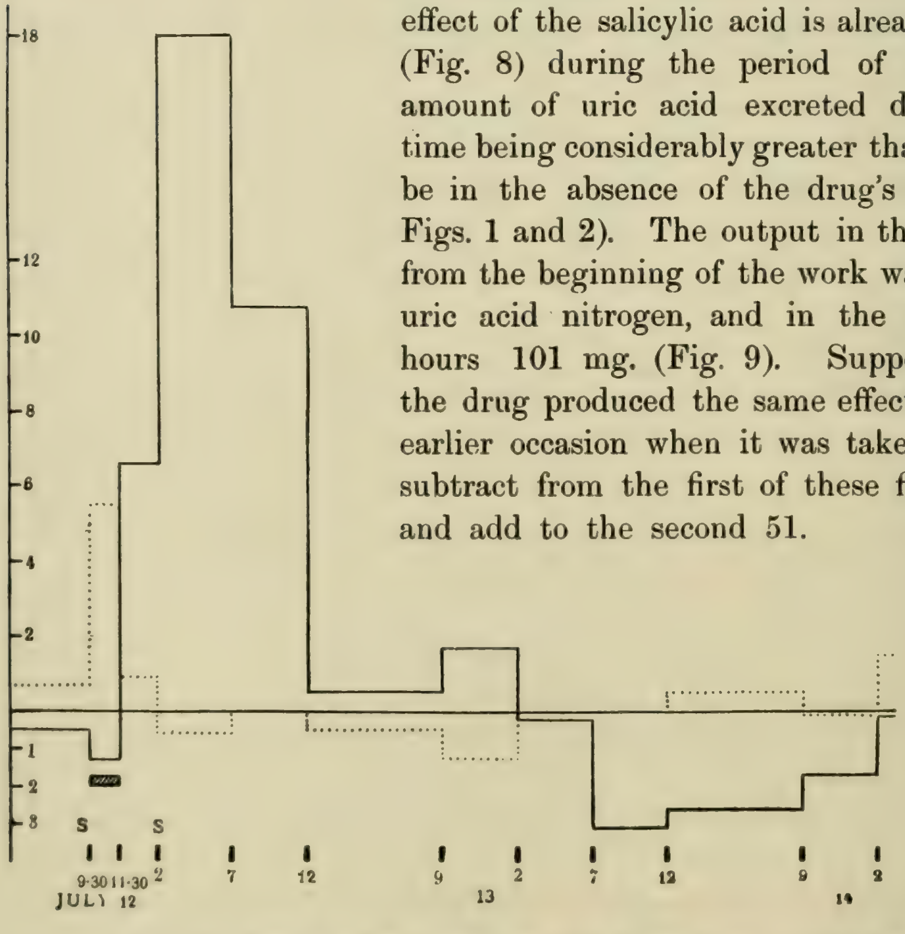

Fig. 8. Exercise (first repetition) and salicylic acid. July 12th.

S. 2 grams salicylic acid taken.

Continuous line. Uric Acid Nitrogen. Dotted line. Purine Base Nitrogen.

Abscissæ represent time in hours.

The base line indicates the normal rate of excretion during each of the portions of the day indicated below. Ordinates represent mg. nitrogen per hour above or below the normal rate of the period.

The duration of the exercise is represented thus ▨.

The observed and the corrected outputs of the experiment of July 12th are placed together in Fig. 9, together with the results of the earlier experiment which gave the data for the correction. The equality in height of two of the columns in this figure is accidental. As was pointed out earlier in this paper (see p. 6), this correction gives figures for the effect of muscular work on July 12th which conform to the progressive changes observable in the effects of the same amount of work on the four occasions on which it was done (Fig. 4); the figures so obtained, that is to say, are less than those for the previous occasion (July 6th) and more than those for the subsequent occasion (July 15th). The figures therefore seem to indicate that the effects of the salicylic acid and of the muscular work were brought about simultaneously, and without either of them being notably modified by the other.

The rates of excretion observed after administration of salicylic acid, in the absence of muscular work, suggests that this drug brings about the excretion of uric acid which would not otherwise have reached the urine, while there is no indication that it increases the processes of formation. If muscular work affected the uric acid output by the same mode of action, it is unlikely that these two agents could act, as they do, simultaneously without interference unless they affect two largely or wholly different sets of tissues. Since we have no evidence at all for such difference in the tissues affected, it is equally admissible to suppose that the modes of action of work and of salicylic acid are entirely different in nature. The result of this experiment is therefore quite compatible with the view that the increased excretion of uric acid which follows muscular work is due to the production of larger amounts of it. Moreover, the output of uric acid during the first 24 hours of this experiment was more than twice as large as that which ordinarily occurs within this time (Fig. 9); this increase is so great that it seems unlikely that the additional output can represent no more than the quantity of uric acid normally kept in stock in the tissues. The most reasonable explanation of these figures seems to be, that a formation of uric acid, greater than that carried on under normal conditions, was in progress. Such increase in the process of formation must have been due either to the exercise or to the salicylic acid, or to both of these. Since other experiments, described in the earlier paper (1) and in the present one, have given evidence of the formation of increased amounts of uric acid as a result of exercise, and since salicylic acid has not been proved to have any such action, the increased production of uric acid indicated by the results of this experiment may be assigned at least in part to the effect of the exercise.

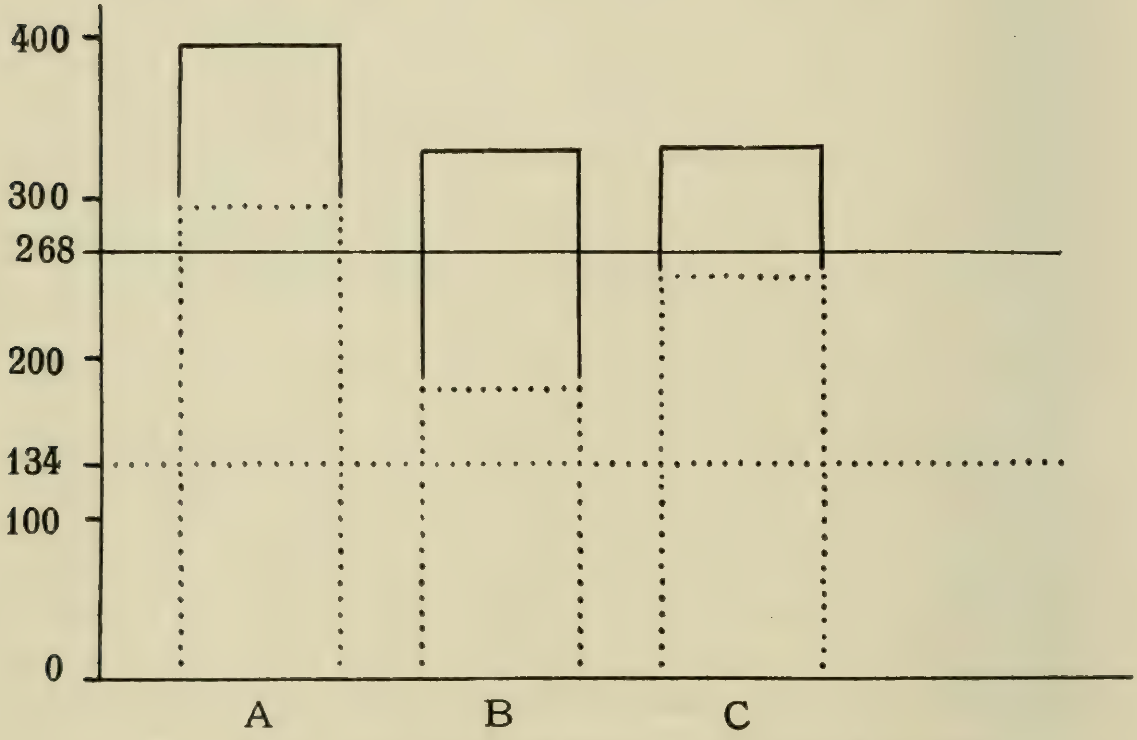

Fig. 9. **Effect of Work and of Salicylic Acid upon Uric Acid output.**

Ordinates represent mg. uric acid nitrogen.
Vertical lines represent 48 hours output.
Dotted portion first 24 hours.
Continuous portion second 24 hours.
Horizontal lines represent average output.
Dotted line, 24 hours (134 mg.).
Continuous line, 48 hours (268 mg.).
A. **July 12th, actual output. Work and salicylic acid.**
B. **July 12th. Corrected output. Work.**
C. **November 25th. Salicylic acid.**

The excretion of purine bases during muscular work.

The low output of uric acid *during* exercise would seem to be due in part to deficient oxidation of the precursors. The purine bases are at this time excreted in such amounts that the total purine content of the urine may differ but little from what is normal (Table I). During work, therefore, there is an alteration in the proportions of the more and the less oxidised forms of a relatively constant amount of purine compounds; one might well suppose that the proportion of the more oxidised form is small, not because any of this substance is retained, but because the other demands upon the supply of oxygen are at this time too great to allow of the formation of larger amounts. The recent observations of Hill and Flack (3) have given evidence that during severe muscular effort "the use of oxygen and production of CO_2 are so rapid in the muscles that the circulation cannot keep pace with the demand." The presence of lactic acid in the urine during exercise, which was demonstrated by Ryffel[1], might also be regarded as indicating the inadequacy of the supply of oxygen. If one considers the total purine excretion, the condition of the urine during muscular work does not yield the support which has been claimed (4) for the opinion, that the low output of uric acid at this time is due to the diversion to the active muscles of blood from organs thought to be important sources of uric acid. To say merely that the output of uric acid is greatly diminished during muscular work is to obscure the evidence afforded by the urine at this time, which evidence shows that it is only the most oxidised purine compound which is diminished. If the view quoted above is to cover the observed facts, one must add to it some supposition to explain the sudden appearance in the urine of a quantity of purine bases greater than has been observed under any other conditions. The source of such an amount of purine bases may be located only in the active muscles, since the other tissues are considered capable at this time of producing only much smaller amounts of the most nearly allied compound. This theory, then, leads one by a process of exclusion to conclude that muscles when active begin suddenly to produce large quantities of purines, since it has been supposed (4) that the muscles are at other times comparatively unimportant sources of purines. It is unnecessary to assume that muscular work produces such a redistribution of the sources of the urinary purines. During a period of work such as that of July 6th (Table I) much the greater part of the total purines of the urine, which in this case were scarcely subnormal in amount, must, in accordance with the consequences of the theory here examined, have been coming from the muscles. It is surely not unreasonable to suppose that, at such a time, the purines excreted were coming to a large extent from their usual sources, of which one of the most important is the muscles; the obvious change which is present is an alteration in the proportions of the different forms of purines. The theory in question is adapted to explain a degree of defect in the total purine content of the urine which is not present in any of these experiments.

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[1] Communication to Physiological Society, October 17th, 1908.

No attempt is here made to contend that the rate of production of purines by the muscles is unaltered during voluntary activity; the increased purine base content of excited muscles was of course shown by Burian (5). What is claimed is, firstly, that the source of a large portion of the purines excreted during exercise must, if one pursues the theory here examined, be placed definitely in the active muscles; and secondly, that there are not sufficient grounds for concluding that this capacity of the muscles becomes comparatively unimportant at other times.

Since it appears that muscles during their activity can produce considerable amounts of purines, it is not necessary to suppose that during the next few hours they can produce such amounts only if they have suffered, as has been suggested (4), actual disintegration of tissue.

The large amount of purine bases excreted during exercise is of interest from another point of view. The diminished output of uric acid which accompanies exercise might be attributed to a difficulty in the excretion of this substance, such as has been supposed to occur under various conditions. If uric acid which was available for excretion had for this reason to be retained in the body at this time, it is unlikely that compounds so closely allied to uric acid as are the purine bases could be excreted in considerable quantities.

Some experiments may now be described which were designed to show whether the oxidation of purine bases during muscular work could be assisted by the inhalation of oxygen. Dr Leonard Hill had suggested that it would be of interest to ascertain whether the effects of muscular work, examples of which had been given in the earlier paper (1), would be altered if the supply of oxygen during the work were increased, and he was good enough to secure facilities for the performance of experiments at the works of Messrs Siebe, Gorman and Co. The exercise was taken by means of an apparatus, constructed for experiments carried out under Dr Hill's direction (6), in which a weight of 60 lbs. is lifted 19½ inches by the hands and one foot. For the purpose of the observations now to be described, a diet of bread, milk, eggs, cheese, butter, and apples was taken in approximately equal amounts daily from October 8th to 31st. The meals were taken at 8.30 a.m., 2.30 p.m. and 7.30 p.m. The average daily output of nitrogen was found to be the same as it had been on the diet of bread and milk, namely 19·7 grams. The first meal was taken as usual on the days of exercise. The times fixed for the collection of urine, and for the exercise, were the same as those adopted in July (see p. 2). On the ninth day after the commencement of the diet (October 16th) work was performed while wearing the Fleuss-Siebe-Gorman dress, designed for use in mines, which delivers two litres of oxygen per minute to the wearer. The weight was lifted 2612 times in the course of two hours. The amount of work done would have been considerably greater but for the fact that the heat of the oxygen inhaled produced during the second hour a good deal of discomfort and retching; this defect in the apparatus is due to the heat evolved by the absorption of the carbon dioxide[1]. The inhalation of oxygen was continued for two and a half hours after the conclusion of the work.

The results are shown in protocol II. The amount of uric acid excreted during the work was as small as it had been on previous occasions when vigorous exercise was taken (cf. Table I); it had been expected that the output would be higher, owing to increased oxidation of the purine bases. The output of purine bases during the work was remarkably low; no explanation of this can at present be offered. During the period following the work, while the inhalation of oxygen was continued, the output of bases rises considerably; it is probable that at this time the supply of oxygen was not as ample as it should have been, owing to mismanagement of the apparatus by the experimenter.

Four days later, on October 20th, work was done without the use of oxygen. The dress, which weighs about 35 lbs., was carried but was not connected with the mouth. The weight was lifted 3400 times. An interval of seven days was then allowed, in order to eliminate as far as possible any improvement in the state of training of the muscles, which would have rendered a comparison of the results obtained with and without the use of oxygen unreliable. On October 28th, oxygen was inhaled during the work and during the next two and a half hours. The dress was modified under Dr Hill's direction in such a way that the arrangement for absorption of carbon dioxide was not utilised, the expired gases passing out into the air; heating of the oxygen was thus avoided. Two 60 cubic foot cylinders of oxygen were used, one during the work and one after it. The weight was lifted 3400 times, as on the occasion when oxygen was not used.

The results obtained show (Table I) that with inhalation of oxygen the output of uric acid is slightly higher, and that of the purine bases slightly lower, than had been the case when oxygen was not used. The difference observed is therefore of the kind which would be expected, but is extremely small in amount. In spite of the inconclusive character of this result, it is difficult to avoid the conclusion that the alterations in the proportions of purine bases and uric acid in the urine during severe exertion are dependent upon the excessive demands made by the active tissues upon the oxygen supplied to the body. This inadequacy of the supply cannot be remedied more effectually than by the inhalation of pure oxygen, yet this proceeding affords one no control over the purposes to which the additional oxygen will be applied. It may well be that, during muscular effort, one cannot introduce into the body an amount of oxygen which will do more than satisfy demands exceeding in urgency any requirement to oxidise purine compounds to the normal extent.

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[1] Since these experiments were carried out, an arrangement for cooling the oxygen has been added to the apparatus.

The suggestions made above as to the oxidation of purines might be followed out in considering the series of observations made in July on the results of exercise. It has been shown that, with repetition of a certain amount of work, the alterations seen during the work in the proportions of the two forms of urinary purines are lessened, and the subsequent output of uric acid becomes subnormal. This change might be attributed to an improvement in the processes of oxidation which convert the purine bases into uric acid, and destroy the latter body; these processes perhaps become adapted to the adverse conditions prevailing during muscular work. This improvement might be such that, in the subsequent period, when the demands upon the supply of oxygen are smaller, the amount of uric acid which would otherwise have been excreted as such, is encroached upon, and the output thus becomes subnormal. This increase in the power of oxidation would perhaps account for the fact that the resort to a new form of exercise did not fully reproduce the series of changes observed on the first occasion. The two sets of untrained muscles presumably reacted to activity in the same way, but in the later case the evidences of this reaction may have been to some extent neutralised by the more active oxidative processes. In this connection, the low output of purine bases on the last few days of the series (see protocol I) is of interest.

One might of course urge with equal force that an increase in the power of oxidation should produce a progressive decrease in the uric acid output during periods of exercise, owing to destruction of larger amounts of this compound; and other such instances, where the evidence is equivocal, are readily found. The uric acid content of the urine presents the net result of the processes of formation and destruction of this substance, which processes constitute a continuous series of oxidative changes. It is obvious that no theory as to the activity at any time of the whole series of changes can be either proved or disproved by appeal to such a net result, since the relative activity of the changes preceding and following the stage of uric acid is unknown.

The diurnal variations in the excretion of endogenous purine bases.

The averages given in the first protocol show that the output is lowest during the hours of sleep. During the morning it is considerably higher; during the afternoon it rises to a still higher level, contrasting in this respect with the excretion of uric acid. During the evening, the output falls to a greater extent than does that of the uric acid, and is at this time about the same as it is during the night. The averages given in the second protocol show that in this series the output was lower in the afternoon than in the morning; these averages are however derived from a much smaller series of estimations. In an earlier series of observations (1) made on the same person, which was of longer duration than either of the two series here described, the output was distinctly higher in the afternoon than in the morning (see Table X in the paper here referred to). It will be observed that the changes undergone by the purine base output in the course of the day are on the whole very similar throughout to those exhibited by the uric acid output (2) (see averages in protocols). The fact that the excretion of purine bases is at its lowest during the night shows that the low rate of excretion of uric acid at this time cannot be attributed to lessened oxidation of the bases.

The relations between the amounts of urine, and of uric acid and purine bases.

If the figures given in the first protocol be examined, it is found that in a series of days or of portions of the day, the rate of excretion of uric acid is on an average greater than the average rate when the volume of urine is below the average volume, and less than the average rate when the volume is above the average; while the excretion of bases varies in the opposite sense. In other words, the rate of excretion of uric acid tends to vary inversely, and that of the bases to vary directly, with the volume of urine (Table II).

In obtaining these averages, periods were excluded when the output of uric acid or of purine bases was markedly affected by exercise or the action of salicylic acid. The figures used were those which gave the normal averages set down in the protocol. The larger series of figures obtained in November 1907 during observations⁽¹⁾ on the same individual was also examined in this way, and yielded a confirmatory result, with the exception that in one of the five periods, namely, that from 2 to 7 p.m., the amount of uric acid was slightly greater when the volume of urine was greater.

The small number of figures contained in protocol II has not been examined.

TABLE II.

Relation between amounts of Urine, and of Uric Acid and Purine Bases.

A. Average rates when volume above average. B. Average rates of all occasions. C. Average rates when volume below average. U.N. Uric Acid Nitrogen. P.B.N. Purine Base Nitrogen. T.P.N. Total Purine Nitrogen.

July 1908.		Volume rate in c.c.	Rates in mg.			
			U.N.	P.B.N.	T.P.N.	
12 to 9 a.m. (19 days)	Rates per hour	**38**	3·46	**1·15**	4·61	A
		32	3·58	1·10	4·68	B
		28	**3·66**	1·07	**4·73**	C
9 a.m. to 2 p.m. (16 days)	Rates per hour	**105**	7·26	**2·88**	**10·14**	A
		81	7·44	2·02	9·46	B
		64	**7·55**	1·51	9·06	C
2 p.m. to 7 p.m. (18 days)	Rates per hour	**122**	6·10	**2·63**	8·73	A
		94	6·64	2·31	8·95	B
		70	**7·26**	1·96	**9·22**	C
7 p.m. to 12 (18 days)	Rates per hour	**64**	5·79	**1·18**	**6·97**	A
		48	5·85	1·10	6·95	B
		41	**5·91**	1·03	6·94	C
24 hours total (19 days)		**1649**	130	**41**	**171**	A
		1352	133	35	168	B
		1054	**137**	28	165	C

The amount of the endogenous purine output.

The figures for the average output of endogenous purines observed in the same person during three different periods are placed together in Table III. In obtaining these averages, days upon which the amounts excreted showed the effects of exercise or the taking of drugs were excluded as far as possible; the days omitted are stated in the protocols. The figures obtained for the amounts of purine bases in November 1907 cannot be introduced into the comparison, owing to a difference in the method of estimation employed.

TABLE III.

	Body weight in kilos.	Average output in mg. Nitrogen					
		Uric Acid		Purine Bases		Total Purines	
		per day	per kilo per day	per day	per kilo per day	per day	per kilo per day
I. November 1907, 24 days	74·9	135	1·80	—	—	—	
II. July 1908, 18 days	72·4	134	1·85	34	0·47	168	2·32
III. October 1908, 10 days	72·8	116	1·59	48	0·66	164	2·25

			Uric Acid	Purine Bases	Total Purines
Coefficients of Variability[1]	of figures giving the above averages	July	7·9	36·0	5·2
		October	13·8	24·7	4·3
	of figures for every day of the series	July, 24 days	28·9	38·0	22·4
		October, 16 days	15·0	23·9	7·8

The average amounts of uric acid excreted daily in the first and second periods are almost exactly the same; during the third period the amount is considerably less. The total purine output, however, is but very slightly lower in the third period than it was in the second (see also Fig. 10). These figures therefore confirm the observations of Burian and Schur (7), which showed that the total amount of purines excreted by a person even at times separated by intervals of months is a remarkably constant quantity, which exhibits smaller variations than does the output of uric acid (see pp. 293, 294 of the paper referred to here). It is obvious that the results of observations in the course of which the uric acid output alone is ascertained are inadmissible in criticism of the statement made by Burian and Schur as to the total purine output; the results of the July and October series brought forward here show how misleading the uric acid output might be if it were assumed to maintain a constant relation to that of the total purines. The conclusion reached by Burian and Schur had reference only to the amounts excreted in 24 hour periods; the existence

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[1] $$\text{Coefficient of Variability} = \frac{\sqrt{\dfrac{\text{sum of squares of deviations from mean}}{\text{number of variates}}}}{\text{mean of variates}} \times 100.$$

See Davenport, *Statistical Methods*, pp. 15, 16. London, 1904.

of diurnal variations has therefore no bearing on the question. They state expressly that they affirm the total purine output to be approximately constant only under uniform conditions of life (p. 303) and within certain wide limits of variation in diet (p. 336); the results obtained in July and October showed that a considerable difference in the uric acid output, with but very slight difference in that of the total purines, may be observed in two periods during which the conditions of life were closely similar, and on diets producing the same total excretion of nitrogen.

Moreover, the total amount of purine compounds excreted upon successive days is relatively constant. If the coefficients of variability of the figures given in the two protocols for the day's output of total purines and of uric acid be taken, it is found that the former figures exhibit the smaller deviations from their average (Table III). The high variability of the amount of purine bases excreted, as expressed by the coefficients, is of course due to the fact that while the absolute amount

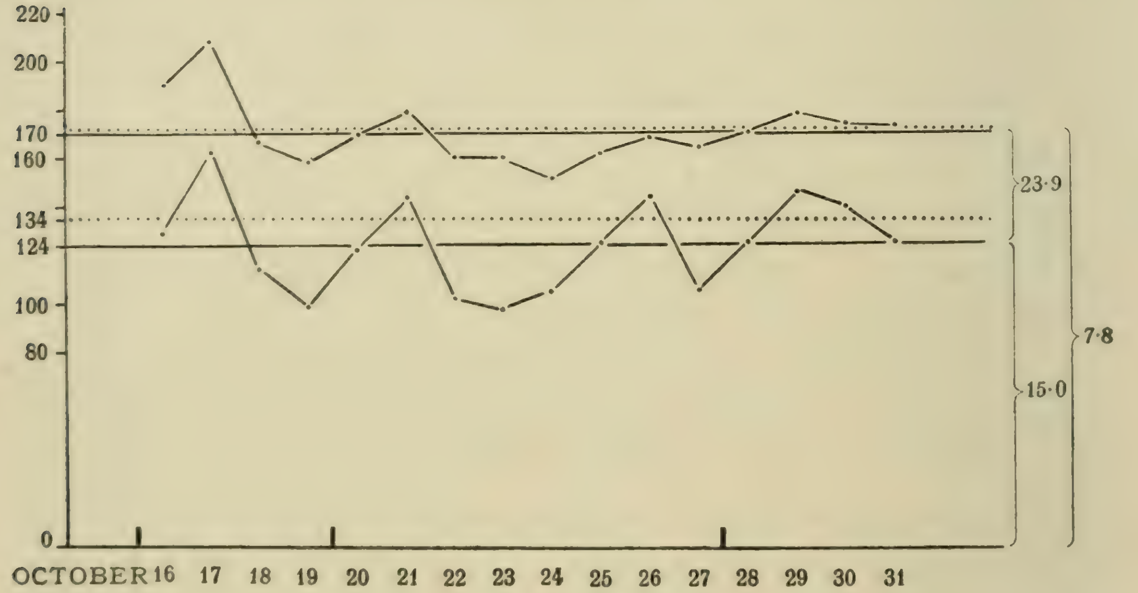

Fig. 10. 24 hours output of Purines. October 1908.

Ordinates represent mg. nitrogen.

Dots joined by lines represent 24 hours output from midnight to midnight; horizontal continuous line represents average of these amounts.

Horizontal dotted line represents average 24 hours output of July series.

Upper three lines. Total Purine Nitrogen.

Lower three lines. Uric Acid Nitrogen.

Vertical lines. Times of exercise.

Figures to right of brackets are coefficients of variability of October series (see Table III).

of these substances is always much smaller than that of the uric acid, they have, as it were, to fill up the variable amounts of uric acid to a more constant level of total purine output (see Fig. 10). The comparative uniformity of the total purine output both in consecutive and non-consecutive periods is illustrated in Fig. 10, which shows the result of each day of the October experiment, together with the averages both of these figures and of those obtained in July. The latter average is based upon the figures for each day of the July experiment, with the exception of the day (July 12th) upon which the combined actions of muscular work and of salicylic acid produced an extremely large output of uric acid; it appeared that this average provided as satisfactory a comparison as could be made between two such series, the more normal days of which gave the averages compared in Table III. An inspection of Fig. 10 shows that the total purine output varied less from day to day than did the uric acid output, while, of the averages obtained in July and October respectively, those of the total purines differ from each other less than do those of the uric acid.

The results brought forward in Table III and Fig. 10 may be regarded as indicating that the subject of these experiments was in October capable of oxidising to the stage of uric acid a proportion of a certain amount of purine compounds smaller than that which could be so ovidised in July of the same year. To judge by the subjective evidence the general health during the later period, when the uric acid output was lower, was distinctly better than during the earlier one, owing to the shorter duration of the experiment, the smaller amount of laboratory work, the more attractive diet, and the cooler weather.

It appears, then, that a relatively constant amount of purine compounds is excreted, while the extent to which these compounds undergo oxidation shows greater variations. These considerations emphasise the importance in investigations on purine metabolism of analyses which give figures for the output of purine bases. The value of such figures is still more evident when one considers that the purine base output varies from the average in a direction opposite to that taken by the uric acid output (1) during muscular work, and in many cases during the next few hours as well (see Table I), (2) with changes in the volume of urine (see Table II), (3) in some forms of fever (8).

Methods of estimation.

Uric acid was estimated by Folin's modification of Hopkins' method; the purine bases were precipitated with silver nitrate in the filtrate from the precipitate of ammonium urate which is obtained in the above method. The nitrogen content of the silver compound of the bases was found by the Kjeldahl method. This method of estimating the purine bases was adopted, because the Camerer-Arnstein method, when applied to the total purines of urine, had given in an earlier series of observations results which in many cases were obviously too low. In some experiments which were made on this subject, it appeared that in this method the uric acid suffers a loss to which the purine bases are not liable. The Camerer-Arnstein method was therefore applied to the urine after the removal of the uric acid. To the figures thus obtained for the amounts of purine bases, a correction was applied corresponding to the amount of uric acid stated by Folin to escape precipitation.

All estimations were made in duplicate.

SUMMARY.

1. During unaccustomed muscular exercise, the output of uric acid is diminished, while that of the purine bases is increased; the total output of purine compounds does not differ greatly from the normal amount. Such exercise is followed by a greatly increased excretion of uric acid.

2. If a certain amount of a certain form of work be performed on a series of occasions, the changes observed show a progressive diminution in amount. The increased excretion of uric acid which followed the exercise on the earlier occasions is replaced by a subnormal output; this alteration cannot be to any great extent due to the exhaustion of an accumulated stock of uric acid, since the resort to a new form of exercise, which employs different muscles, may be followed by the excretion of an amount considerably greater than is normal. The results of repetition of a single form of exercise, and of the substitution for it of a new form, are therefore not favourable to the theory that the increased excretion of uric acid which follows exercise is due to the sweeping out of an amount of it which is present as such in the body, and would otherwise have been retained there; these results indicate rather that exercise is followed by heightened activity of the processes by which uric acid is produced.

3. Salicylic acid and muscular work can produce their characteristic effects upon the output of uric acid simultaneously without any apparent modification, and the amount excreted under these conditions

is extremely large. These facts are most readily explained by supposing that muscular work leads to an increased formation of uric acid.

4. The increase of purine bases and decrease of uric acid in the urine during muscular work appear to be due to defective oxidation of purine compounds. It was not found possible to produce evidence of a definite increase in these oxidative processes by inhalation of oxygen.

5. The total amount of purine compounds excreted is a more constant quantity, both from day to day and at periods separated by intervals of months, than is the output of uric acid.

6. The output of purine bases exhibits variations, corresponding to different portions of the day, which are very similar to those exhibited by the uric acid output.

7. The amount of uric acid excreted tends to vary inversely, and that of the purine bases to vary directly, with the volume of urine.

In conclusion, I wish to express my great indebtedness to Dr Leathes for assistance throughout this investigation. My thanks are due also to Dr Hill for his supervision of the experiments on the effect of oxygen inhalation, and to Messrs Siebe, Gorman and Co. for the ample facilities which they gave for these experiments.

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PROTOCOL I.

Vol. = volume of Urine in c.c. U.N. = mg. Uric Acid Nitrogen. P.B.N. = mg. Purine Base Nitrogen. T.P.N. = mg. Total Purine Nitrogen.

The figures under the headings of different portions of the day indicate rates per hour during the period.

On the 6th, 12th, 15th, 18th, and 20th, the urine was collected between 12 and 9.30 a.m., 9.30 a.m. and 11.30 a.m., 11.30 a.m. and 2 p.m. The rates during these periods are given under the headings of the usual portions of the day.

a, Between 9.30 and 11.30 a.m. two 10 lb. weights lifted from floor to above head 850 times. *b*, 2 grams salicylic acid taken at 9 a.m. and 2 a.m. *c*, New exercises from 9.30 to 11.30 a.m. *d*, Walk, 9.30 a.m. to 6.45 p.m.

1908	12 to 9 a.m.				9 a.m. to 2 p.m.				2 p.m. to 7 p.m.				7 p.m. to 12				24 hrs. Total output.				
	U.N.	P.B.N.	T.P.N.	Vol.	U.N.	P.B.N.	T.P.N.	Vol.	U.N.	P.B.N.	T.P.N.	Vol.	U.N.	P.B.N.	T.P.N.	Vol.	U.N.	P.B.N.	T.P.N.	Vol.	
July 1	3·91			24	10·11			69	5·29			63	5·69			40	141			1082	1
2	3·52	1·20	4·72	26	6·99	1·10	8·09	70	5·92	3·92	9·84	83	4·60	1·37	5·97	38	119	43	162	1186	2
3	3·63	1·42	5·05	26	9·22	1·67	10·89	49	7·49	1·21	8·70	45	7·29	0·45	7·74	40	153	29	182	905	3
4	3·94	1·05	4·99	25	6·77	3·46	10·23	54	5·29	4·34	9·63	86	4·99	1·28	6·27	40	121	55	176	1127	4
5	3·90	1·46	5·36	29	6·30	3·64	9·94	112	6·96	1·64	8·60	91	6·46	1·11	7·57	46	134	45	179	1510	5
a 6	3·78	1·16	4·94	31	1·39 4·83	7·76 7·83	9·15 12·66	27 19	13·06	1·19	14·25	42	12·81	0·25	13·06	40	180	53	233	811	6
7	6·22	0·62	6·84	22	13·55	0·64	14·19	41	12·92	0·90	13·82	68	8·76	0·38	9·14	35	232	15	247	917	7
8	4·81	0·46	5·27	23	6·70	2·29	8·99	86	4·49	2·84	7·33	135	6·18	1·18	7·36	47	130	36	166	1565	8
9	4·34	0·49	4·83	30	6·74	1·48	8·22	71	8·73	0·73	9·46	77	5·28	0·54	5·82	37	143	18	161	1195	9
10	3·68	1·00	4·68	31	6·02	4·66	10·68	110	6·19	2·54	8·73	155	6·52	0·41	6·93	58	127	47	174	1888	10
11	3·61	0·81	4·42	36	9·15	0·96	10·11	91	4·14	3·52	7·66	124	5·12	1·01	6·13	52	128	35	163	1655	11
a, *b* 12	3·07	1·81	4·88	30	6·17 14·05	7·48 2·89	13·65 16·94	36 30	24·70	1·71	26·41	92	16·61	1·09	17·70	62	283	54	337	1207	12
13	4·12	0·60	4·72	33	9·15	0·78	9·93	42	6·44	2·37	8·81	57	2·76	1·11	3·87	44	129	27	156	1010	13
14	0·98	1·65	2·63	29	5·78	1·60	7·38	80	6·48	3·78	10·26	122	3·92	2·35	6·27	80	90	54	144	1670	14
a 15	3·20	1·72	4·92	42	1·59 3·73	4·03 4·02	5·62 7·75	62 46	5·55	3·11	8·66	130	5·61	2·21	7·82	84	99	61	160	1708	15
16	3·41	1·30	4·71	36	6·96	2·80	9·76	100	5·61	3·90	9·51	117	6·30	0·68	6·98	65	125	49	174	1730	16
17	3·23	0·82	4·05	38	8·68	1·67	10·35	57	8·94	0·91	9·85	78	4·18	1·89	6·07	43	138	30	168	1129	17
a 18	3·65	1·01	4·66	29	2·85 2·02	4·59 4·25	7·44 6·27	51 36	4·00	3·28	7·28	86	4·84	2·21	7·05	83	90	57	147	1313	18
19	2·18	1·86	4·04	35	7·72	1·41	9·13	78	9·49	1·16	10·65	113	6·57	0·63	7·20	59	138	33	171	1566	19
c 20	3·85	0·68	4·53	36	5·35 5·40	6·06 5·27	11·41 10·67	57 98	6·46	0·68	7·14	103	6·28	1·76	8·04	75	124	44	168	1592	20
21	4·05	1·40	5·45	45	8·45	2·91	11·36	132	5·77	2·96	8·73	122	4·96	1·37	6·33	52	132	49	181	1934	21
22	4·03	0·85	4·88	30	8·19	1·52	9·71	64	5·51	2·22	7·73	112	5·42	1·06	6·48	46	131	32	163	1400	22
23	3·30	0·89	4·19	26	7·46	0·97	8·43	76	7·20	0·74	7·94	98	5·93	0·34	6·27	49	131	18	149	1337	23
d 24	3·03	0·56	3·59	21	6·55	1·73	8·28	35	7·65	1·05	8·70	34	7·59	0·52	8·11	50	136	22	158	786	24
25	4·65	0·34	4·99	25	7·91	0·25	8·16	38	8·19	0·58	8·77	41	7·91	0·68	8·59	39	162	11	173	816	25
26	5·54	0·29	5·83	26																	
Averages all days	3·75	1·02	4·77	30	7·29	2·73	10·02	67	7·70	2·14	9·84	91	6·50	1·08	7·58	52	141	38	179	1321	
Averages normal	Omit 1, 7, 8, 14, 25, 26				Omit 1, 6, 7, 12, 13, 15, 18, 20, 24				Omit 1, 6, 7, 12, 15, 20, 24				Omit 1, 6, 12, 13, 14, 24, 25				Omit 1, 6, 7, 12, 14, 15, 18				
days	3·58	1·10	4·68	32	7·44	2·02	9·46	81	6·64	2·31	8·95	94	5·85	1·10	6·95	48	134	34	168	1352	

PROTOCOL II.

U.N., P.B.N., T.P.N., Vol., as in Protocol I.

On the days of exercise (16th, 20th, 28th) the times for the collection of urine were the same as on the similar days of the July series (see Protocol I).

a, *b*, *c*. Exercise 9.30 to 11.30 a.m. (60 lb. lifted 19½ inches). *a*. Weight lifted 2612 times. Oxygen inhaled from 9.30 a.m. to 2 p.m. by Fleuss-Siebe-Gorman apparatus. *b*. Weight lifted 3400 times. *c*. Weight lifted 3400 times. Oxygen inhaled from 9.30 a.m. to 2 p.m. by different apparatus (see p. 17).

1908	12 to 9 a.m.				9 a.m. to 2 p.m.				2 p.m. to 7 p.m.				7 p.m. to 12				24 hrs. Total output				
	U.N.	P.B.N.	T.P.N.	Vol.	U.N.	P.B.N.	T.P.N.	Vol.	U.N.	P.B.N.	T.P.N.	Vol.	U.N.	P.B.N.	T.B.N.	Vol.	U.N.	P.B.N.	T.P.N.	Vol.	
Oct. *a* 16	2·87	2·02	4·89	50	1·44 4·90	1·79 6·63	3·23 11·53	46 34	8·96	2·94	11·90	56	8·18	1·70	9·88	44	128	62	190	1199	16
17	4·78	0·97	5·75	30	9·64	2·90	12·54	73	8·33	2·47	10·80	69	5·84	1·83	7·67	46	162	45	207	1210	17
18	3·81	1·50	5·31	29	8·40	3·13	11·53	109	4·29	2·57	6·86	70	3·28	2·07	5·35	39	114	52	166	1352	18
19	2·38	1·68	4·06	25	6·16	3·86	10·02	107	5·28	3·07	8·35	60	4·08	1·93	6·01	41	99	59	158	1265	19
b 20	2·74	1·10	3·84	33	3·07 6·36	4·74 3·51	7·81 9·87	52 72	7·92	1·61	9·53	50	6·80	2·41	9·21	48	122	49	171	1085	20
21	3·75	0·68	4·42	24	9·68	2·18	11·86	67	6·76	2·40	9·16	65	5·56	1·15	6·71	52	144	35	179	1142	21
22	3·18	1·09	4·27	22	6·35	4·99	11·34	100	5·28	2·61	7·89	87	3·03	1·66	4·69	39	103	56	159	1350	22
23																	98	61	159	1310	23
24																	105	46	151	1410	24
25																	126	36	162	1180	25
26																	144	24	168	1220	26
27																	106	58	164	1220	27
c 28	3·69	1·20	4·89	33	4·96 6·96	4·39 2·15	9·35 9·11	44 54	6·92	1·91	8·83	50	5·26	2·00	7·26	43	125	45	170	1006	28
29	4·02	1·58	5·60	30	9·87	1·02	10·89	60	7·23	1·44	8·67	62	5·15	1·36	6·51	43	146	33	179	1085	29
30																	140	35	175	1350	30
31																	127	47	174	1100	31
Averages all days	3·47	1·31	4·78		7·02	2·87	9·89		6·74	2·36	9·10		5·44	1·78	7·22		124	46	170	1218	
					Omit 16th, 20th, 28th												Omit 16th, 17th, 20th, 21st, 28th, 29th				
					8·23	2·48	10·71										116	48	164		

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A Preliminary Note on the Examination of the Fat in the Liver in Health and Disease.

By E. L. KENNAWAY and J. B. LEATHES.

FOR many reasons special interest attaches to the combinations of fatty acids found in the liver. As a general rule the amount of fatty acids present in this organ is found to be comparable with that found in the heart or kidneys, and it is often less. But under exceptional circumstances in animals that are apparently normal, and not only in those that are diseased, the amount is excessive and then may be very much greater than the largest amounts ever found in the heart or kidney. In pathological conditions in man 60 per cent. and 70 per cent. of the dry solid matter of the liver may be fat, and Rosenfeld has found such amounts too in dogs that have been treated with phlorizin in starvation. There is no other organ in the body in which anything like such large amounts of fat ever occur. Moreover, as was shown by Hartley and Mavrogordato,[1] when these large quantities of fat occur in the liver the fatty acids are not those which normally characterize the fats and lipoids of the liver and other viscera, highly unsaturated acids with an iodine value of about 130, but consist largely of the saturated acids and oleic acid, and therefore absorb in extreme cases hardly more iodine than the acids that can be obtained from the fat of adipose tissue. This and other facts suggest, as has been pointed out before, that the liver stands in a different relation to the fats of the body from all other organs, and that fat which is put into circulation from the connective tissues undergoes some preliminary treatment in this organ before it is distributed to the other viscera in which it is finally oxidized completely.[2] This preliminary treatment would seem to consist in part of changes in the fatty acids whereby they are converted into those less saturated acids which are characteristic of the fat in the working organs, but which are generally absent from fat stored in adipose tissue. Since it has been repeatedly found that lecithin and other phosphatide lipoids from various sources yield on saponification fatty acids with a high iodine value, it is possible that this change in the character of the fatty acids occurs only after the acids have been built up into these more complex combinations. It is known that phosphatide fats are present in the liver in considerable amounts; they constitute frequently a half, or perhaps more than a half, of the fat that the organ contains. And it may therefore be a special function of the liver to build up out of the connective tissue fat which reaches it, in the first instance lecithin and the related lipoid substances, for the use not only of its own cells but it may be for those in other organs as well, and the desaturation of the acids may occur only after they have entered the lecithin molecule and have come within the sphere of influence of the phosphoric acid and other groups which the lipoids contain, but which ordinary fats do not. One of the first points which suggest themselves for investigation, therefore, is a comparison of the nature of the fatty acids that occur in the simple glycerides present in a normal liver, with that of the acids occurring in combination with phosphorus and nitrogen. The present communication contains the results of some preliminary experiments on these lines, which form part of a study of the functions of the liver in relation to the metabolism of fat in health and disease.

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[1] P. Hartley and A. Mavrogordato, *Journ. Path. and Bact.*, 1908, xii, p. 371.

[2] Leathes, "Problems in Animal Metabolism," 1906, p. 118,

In the first place it was necessary to effect some separation of the different combinations of fatty acids present in the liver. For this purpose the livers were minced and treated with alcohol; the alcohol was strained off after twenty-four hours and the solid matter pressed in a screw press. This process having been repeated several times the combined alcoholic extracts were evaporated *in vacuo* at a temperature not above 50 C. while a slow stream of carbonic acid passed through the fluid. In some cases the extraction with alcohol was followed by treatment with ether, which was then evaporated in the same way. The oily residue left after complete removal of the alcohol or ether was taken up in dry ether and the solution filtered. By the addition of two or three volumes of acetone the first separation is carried out; the acetone precipitating the lecithin and other phosphatides. The acetone solution evaporated in the same way as the original extracts leaves a residue consisting for the most part of simple glycerides with some cholesterin and a little unprecipitated phosphatide. This residue treated with dry acetone dissolves for the most part, and the filtered solution evaporated to dryness leaves a residue soluble in petroleum ether, in which solvent it is best kept for examination. The acetone precipitate dissolved in ether and filtered should be reprecipitated with acetone repeatedly till everything soluble in acetone is removed. The final ether solution is then treated with two or three volumes of absolute alcohol, when a precipitate is obtained in which jecorin will be present, while lecithin remains in solution. The alcoholic solution evaporated down leaves a residue which is taken up in absolute alcohol and may be kept dissolved in that solvent or else in petroleum ether. The alcohol precipitate reprecipitated from ether by means of alcohol and washed with alcohol can if protected from moisture be preserved as a dry powder.

In this way the substances soluble in ether are divided into three fractions: (1) a fraction composed mainly of simple glycerides with some cholesterin, soluble in acetone; (2) a fraction soluble in alcohol which may be referred to as the lecithin fraction; and (3) a fraction insoluble in alcohol, the jecorin fraction.

The three fractions obtained in this way from the liver of pigs (two series), from a dog, and from a goat have been examined as follows: a weighed amount, about 1 grm., of each of the fractions was treated with potash and alcohol on the water bath for one to two hours. The soap solution obtained was washed with hot water into a Liebermann flask, made acid with sulphuric acid and shaken with a known volume of petroleum ether; and after the separation of the two fluids had taken place a fraction of the petroleum ether solution removed, evaporated to dryness, at first in a stream of carbonic acid and then *in vacuo* over the water bath. The weight of the residue gives the percentage of fatty acid, contaminated it may be with some cholesterin, contained in the sample. The weighed fatty acids serve then for a determination of the iodine value.

The acid aqueous fluid left in the Liebermann flask after removal of the petroleum ether can be used for determination of nitrogen and phosphorus, only in that case any precipitate that there may be, insoluble both in aqueous acid and in petroleum, must be oxidized too, since it contains both nitrogen and phosphorus. During the saponification small quantities of nitrogen escape as ammonia. This was caught in titrated sulphuric acid, the heating with alkali being conducted in a stream of hydrogen. This procedure, though complicated, presents certain advantages over the more direct combustion of separate portions of the original fatty materials in Kjeldahl flasks which, owing to the large amounts of fatty acids contained in the material analysed, requires care and long heating. In some cases the latter method was employed, in most the former.

In Table I are given, for comparison with our results in Table II: (1) The percentage of fatty acids and the iodine value of these acids obtained from the adipose tissue fat of the species of animals to which the data in Table II apply; (2) figures relating to the composition of lecithin of the theoretical formula containing two stearyl radicles, to that of a lecithin obtained by Erlandsen from the bullock's heart muscle, and to that of three preparations obtained by Baskoff from the liver of the horse; and (3) similar figures for the preparations of jecorin analysed by Drechsel and by Baskoff.

The figures given in Table II, the results of our work on the lines described above, show, as we expected, that we were seldom if ever dealing with completely isolated chemically pure substances, but they show that a separation has been effected which is sufficient for the solution of the question propounded in the early part of this communication. The preparations soluble in acetone, referred to in the first group of columns of the table, were, to judge from the percentage of fatty acids which they contained, much nearer to simple glycerides than to lecithin or jecorin. But since the extracts of the liver may have contained free fatty acids, and certainly would contain some cholesterin, and the presence of these in any considerable amount would tend to raise the figure given in this column for the percentage of fatty acids obtained on saponification by the methods used, a truer indication of the degree to which the simple glycerides of fatty acids have been separated from complex lipoids in these preparations is given by the nitrogen and phosphorus content. This shows that they cannot have contained, at any rate, more than from 10 per cent. to 20 per cent. of lecithin or jecorin. And, therefore, the iodine values of the preparations in the first column show clearly that the highly unsaturated acids in the liver are not confined to the phosphatides. For instance, if the iodine value of 120 obtained in the preparation from the pig's liver No. 1 were due to a mixture consisting of 20 per cent. of fatty acids from lipoids and 80 per cent. of fatty acids such as occur in the adipose tissue of pigs, with the iodine value 55, then the iodine value of the acids mixed with these latter would have to be 380, which is so much greater than that found for the fatty acids obtained from the other fractions, that it is certain that the simple glycerides occurring in the pig's liver, if they have come from the animal's adipose tissue, must have been changed in character and been converted into acids which are less saturated. The highly unsaturated acids, then, are not confined in the liver to the compounds containing phosphorus. These latter compounds, however, to judge from the figures in the table, contain acids which, generally speaking, are more unsaturated than those in the form of simple glycerides. This would be the result to expect if fatty acids when they arrive in the liver are of the type that occurs in the adipose tissue and, generally speaking, in the food, and then in the liver they undergo a process of desaturation: so that the preparations dealt with in the first column represent fat in process of being desaturated, partly still unchanged, partly already changed, whereas the fatty acids in the second and third columns are in a later stage more completely desaturated and further combined up with phosphoric acid and other groups into the lipoid molecules of "lecithin" and "jecorin."

The iodine values are, it must be noted, in all cases minimal values. That this is so is shown, for instance, by the following facts:—

(1) As a general rule, as soon as a liver was received for examination a portion of the fresh-pulped tissue was heated with potash and alcohol in order to estimate the total amount of fatty acids present in all combinations, and the iodine value of the mixed total acids so obtained was determined. Among the human livers, for instance, No. 1 contained 3·45 per cent. of its weight of higher fatty acids with the iodine value 135·6; No. 2 contained 3·2 per cent. with the iodine value 134·5. The mixed acids from all sources obtained in this way had been exposed to a much less prolonged treatment than those obtained from the different preparations of the several kinds of fat, glyceride, lecithin, or jecorin, after they had been separated from one another. In the case of the former the iodine value was determined within forty-eight hours of removal from the body; before this could be done in the case of the latter three weeks or more had elapsed. Accordingly the former—*i.e.*, the mixed acids—have a higher iodine value not only than those from the fat soluble in acetone but also than those obtained from the lecithin fraction. This was done, too, in the case of the liver of pig No. 2. The preliminary examination gave in this case 3·15 per cent. of higher fatty acids with the iodine value of 123·1, which is higher than that found subsequently for the acids from the isolated lecithin fraction, and less only than that for the jecorin fraction, which accounted for not more than, at the outside, 5 per cent. of the fatty acids present in the liver.

(2) But even the iodine value obtained for acids prepared by saponifying fresh liver tissue is lower than the real value, for the heating with potash and alcohol causes some changes in the acids. Thus a preparation of "lecithin" was treated with Wijs's iodine solution in the usual way for the determination of the iodine value and absorbed 83·1

per cent. At the same time another portion of the same preparation was saponified and the percentage of fatty acids contained in it determined: 60·5 per cent. were found and their iodine value was now 120·7. If the unsaponified lecithin had the iodine value 83·1, and contained 60·5 per cent. of fatty acids, these acids must have had the iodine value 137·3. This value had fallen to 120·7 during saponification.

The iodine values given in the table are therefore minimal values, and the argument as to the desaturation of the fatty acids present in the form of glycerides is stronger even than the figures show.

Other points brought out in the table that seem to call for comment even at this stage of the inquiry are the following:—

The lecithin fraction gives figures which do not agree with those for lecithin of the usually supposed constitution. The percentage of fatty acids was always less than required by the accepted formula, as was also the phosphorus; the nitrogen, on the other hand, was higher. Four preparations from the pig's liver No. 1 were analysed: those from the second alcoholic extract, from the third, from the fourth and fifth mixed, and fourthly from a preparation obtained by mixing the lecithin fractions of all eight extractions and reprecipitating this mixture three times with acetone. The percentage of fatty acids in these preparations, omitting the analysis of that from the third extraction which was technically faulty, was remarkably constant, ranging from 62·2 to 63·8. The ordinary formula for lecithin requires about from 68 per cent. to 71 per cent., according to whether palmitic, stearic, or oleic acid is supposed to be present. The figures obtained from the liver of pig No. 2 were lower still; so, too, were those from the dog's liver. Baskoff from the liver of the horse obtained lecithin containing 62·5 per cent. and 63·6 per cent. of fatty acids. The percentage of nitrogen found by him was 2·7, higher still than ours, and that of phosphorus, 3·5 per cent., though higher than our figures, is lower than that required by the accepted formula—3·8 per cent.

The constancy in a number of preparations of these variations from the accepted figures for lecithin may be interpreted as meaning that the lecithin in the liver has a different composition, or, as is perhaps more likely, that the mode of separation adopted fails to separate it from some other phosphatide as yet unknown. The figures for the lecithin from the liver of pig No. 2, showing a still greater departure from the true lecithin figures, lower percentage of fatty acids, lower phosphorus, and higher nitrogen content, would in that case mean that this unknown phosphatide was relatively more abundant in this liver.

The jecorin fraction gives the test for jecorin, reduces cupric oxide, gives a red colour with silver and ammonia, and after hydrolysis an osazone melting at 203° C. The fatty acids present in the preparations from the pigs' livers were fairly constant in amount: ten estimations were carried out on different samples from pig No. 1, two of them being by a different method; three were technically faulty; the seven others ranged between 57·7 per cent. and 53·6 per cent. Those carried out on the final preparations obtained by mixing all the earlier ones and reprecipitating once with acetone and once with alcohol gave 54·3 per cent. and 54·4 per cent. The preparation from pig No. 2 gave the figure 55·0.

The figures given by Baskoff for preparations of jecorin from the liver of the horse are 40·75 per cent. (p. 410) and 37·85 per cent. But since our preparations of jecorin fractions from the dog and the goat give again very different figures in each case, it seems reasonable to expect to find that substances of quite different constitution appear in this fraction according to the species of animal from which it is obtained.

The iodine value of the fatty acids, as pointed out before, is necessarily an understatement of the degree to which they are unsaturated. The preparations from the fourth and fifth and the sixth and seventh extracts of the livers from which the figures in the table marked pig No. 1 were obtained, after being mixed and twice reprecipitated, yielded acids with an iodine value of 133, which at an earlier stage had absorbed from 140 per cent. to 150 per cent. of iodine. And even at this earlier stage the preparations had undergone much manipulation of the same kind as that which resulted in this change. But the high figures given by these preparations seem to show clearly that the most highly unsaturated acids are found in this fraction.

The nitrogen and phosphorus estimations carried out on different preparations from the pigs' livers agree fairly among themselves. The nitrogen figures are lower than those given by Drechsel and by Baskoff for the jecorin from the horse's liver. The phosphorus figures fall within the limits of variation in Drechsel's analyses. The single preparations from the dog and the goat require confirmation, but seem to show differences from the jecorin of the pig or horse like the percentage of fatty acids.

Table I.

Simple Glycerides from Adipose Tissue.

In the	Contain fatty acids, per cent.	Having the iodine value
Dog	about 95	about 60
Pig	,,	,, 55
Goat	,,	,, 40
Man	,,	,, 65

Lecithins.

	Fatty acids per cent.	Having the iodine value	Nitrogen per cent.	Phosphorus per cent.
Theory for distearyl-lecithin	70·4	—	1·73	3·84
Erlandsen[1] (bullock's heart)	70·5	110	1·79	3·95
Baskoff[2] (horse's liver) (1)		—	2·70	3·50
,, ,, (2)	—	—	1·95	4·00
,, ,, (3)	—	—	3·90	3·40

Jecorin (Horse's Liver).

	Fatty acids per cent.	Nitrogen per cent.	Phosphorus per cent.
Drechsel	—	2·86	3·2 to 3·7
Baskoff[2]	40·75 37·85	2·52 to 3·1	2·8 ,, 3·0

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[1] Erlandsen: *Zeitschrift für Physiologische Chemie*, 1907, li, p. 71.

[2] Baskoff: *Ibid.*, 1908, lvii, p. 395. The different preparations of lecithin analysed by Baskoff were obtained (1) from the alcoholic extract of the liver; (2) from the ethereal extract; and (3) from an alcoholic extract obtained after extraction with ether.

TABLE II.—SUBSTANCES EXTRACTED FROM THE LIVER BY ALCOHOL (SOLUBLE IN ETHER).

	SOLUBLE IN ACETONE				INSOLUBLE IN ACETONE							
	Simple glycerides (+ cholesterin)				Soluble in alcohol ; lecithin fraction				Insoluble in absolute alcohol ; jecorin fraction			
	Fatty acids per cent.	Iodine value	Nitrogen per cent.	Phosphorus per cent.	Fatty acids per cent.	Iodine value	Nitrogen per cent.	Phosphorus per cent.	Fatty acids per cent.	Iodine value per cent.	Nitrogen per cent.	Phosphorus per cent.
Dog	84·8 ± 0·2	97·0	0·69	0·53	57·2	105·5	2·02	3·35	35·4	103·0	2·54	2·90
		±0·1	±0·02	±0·025			2·03				±0·00	±0·12
					(2)							
					62·2	125·7	2·06	3·25	(4 and 5)			
Pig (1)	88·4	116·1	0·31	0·61	±1·0	±0·3	±0·00	±0·08	55·5			
	88·6	120·1	±0·01	±0·01	(3)		1·92	3·08	57·7	150·2	2·07	3·15
								±0·09			±0·05	
					(4 and 5)				(6 and 7)			
	90·1	114·9	0·35		63·8	130·0	[2·52]	3·19	56·0			
	92·7	106·0	±0·01			±2 0		±0 04				
					Mixed and reprecipitated three times				53 6	145·2	1·72	3·32
									53·8	148·3	±0·02	0·08
					62·3	128·7	2·33	3·76	Mixed and reprecipitated twice			
						±1·0	±0·02	0·14				
									54·3	122·9	2·09	3·29
									54·4	123·1	±0·00	±0·12
,, (2)	96·2	75·8	0·36	0·23	57·3	106·1	2·49	3·04	55·5	130·0		
	95·0	77 6	±0·00	±0·01	56·5	108·6	2·41	3·10		±2·8		
Goat	94·2	87·5	0 37	0·14					19·46	104·0	3·81	3·00
	92·4	90·0	±0·02	±0·01					19·38	103·6	±0·02	±0·00
Man (1)	87·5	123·4			57·1	116·8						
					58·8	117 0						
,, (2)	88·1	110·5			60·4	96·1						
,, (3)	(*a*) 89·4	101·9			57·0	115·1			54·2	[110·0]		
	(*e*) 97·1	69·6										
,, (4)	96·2	89·9			58·3	71·6			48·8	88·5		
	95·0	89·9										

Of the human livers No. 1 was from a case of diabetes mellitus, No. 2 from a case of carcinoma of the breast; neither of these was "fatty," and the latter was free from carcinomatous deposits. No. 3 was from a case of hæmorrhagic pancreatitis; it contained 8·54 per cent. of fatty acids, with the mean iodine value 85·7; it was extracted four times with alcohol; this extract (*a*) contained 18·8 per cent. of fatty acids in forms soluble in acetone and 6·6 in forms that were insoluble in acetone, of which 5·3 per cent. were in forms soluble in alcohol and 1·3 insoluble. This liver was then extracted twice with ether, and this extract (*e*) was almost entirely soluble in acetone and contained 33·5 per cent. of fatty acids. No. 4 was from a case of mitral disease and was also fatty, containing 8·1 per cent. of fatty acids with the iodine value 87·9.

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ON THE PRESENCE OF HÆMOLYTIC FACTORS IN MILK.[1]

By JANET E. LANE-CLAYPON, M.B., D.Sc., *Jenner Research Student, Lister Institute of Preventive Medicine, London.*

A GOOD deal of work has been done in relation to the transmission of immunity by milk.

Ehrlich (1892) showed that a rapid and high degree of immunity to ricin, abrin, robin, and tetanus toxin was conferred by suckling, where the mother was highly immune to those bodies. Later with Brieger (1893), and also with Wassermann (1894), he showed that diphtheria antitoxin passes out in milk in considerable quantities, being present in a concentration of about $\frac{1}{15}$ to $\frac{1}{30}$ that of the blood. Vaillard (1896), however, failed to trace the passage of tetanus, anthrax, and cholera antitoxins in the milk. Kraus (1901) found immune agglutinins, but Remlinger (1899) failed to trace normal agglutinins. Moro (1901) showed that the bactericidal power of the blood of breast-fed infants increased after birth, but fell rapidly on subsequent artificial feeding; he failed to trace any bactericidal substances in the milk, and concluded they were present in a masked form. Moro (1901) and Kraus (1901) failed to detect the presence of complement in milk; this last observer, working with immune sera, failed to detect any immune body. Bulloch (1902) obtained hæmolysis on adding complement to the milk of an immunised animal. The immune body was present in about one-third amount of that of the blood. Bertarelli (1906) obtained similar results, also finding smaller quantities of immune body in the milk than in the blood. Pfaundler and Moro (1907) obtained definite evidence of complement in milk, but they failed to find any normal amboceptor. The failure to find a complement on the previous occasion was due to having used insufficient quantities. A full résumé of the literature is given by Pfaundler (1908).

On the whole, therefore, although there is some discrepancy, it appears that there is considerable evidence in favour of both amboceptor and complement being present in milk, some observers having recorded one and some the other.

Since Bulloch and Bertarelli both detected immune body in milk, it seemed probable that normal amboceptor would also be present, and that the failure to trace it might be due to the peculiarity of ox serum discovered by Bordet (1906), namely, that a third factor, "ox colloid," is essential, in addition to amboceptor and complement. By the application of this discovery I have been able to demonstrate the presence of amboceptor in milk, and thus to confirm Bordet in his deductions.

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[1] Received June 28, 1908.

METHOD.

The milk was used four to six hours after milking; if obtained from a shop in the ordinary way the complement is almost entirely absent. Guinea-pig cells were selected, as they are readily hæmolysed by ox serum. Ox serum heated for half an hour at 56° C., was used as a solution of amboceptor. A mixture of guinea-pig cells, ox serum 56° C., and fresh guinea-pig serum, was used as the control hæmolytic system.

In using whey it is necessary to exercise a little care: if the whey be allowed to stand long on the curd after clotting has occurred the complement can no longer be traced; it appears to be either destroyed or in some way masked by the curd. On the other hand, if the clot be not allowed to contract thoroughly the amboceptor does not appear to be expressed with the whey, since, if the curd remains very granular (as it does in some cow's milk) the amboceptor can be traced in the curd, and can be extracted by washing. As a rule, I found that if 100 c.c. of milk be raised to 40° C. on the water-bath and 0·3 c.c. of Crosse and Blackwell's rennet be added, clotting takes place in a very few minutes. The clot may be left for ten to twenty minutes, but should then be broken up when it contracts readily and squeezes out the whey.

The "ox colloid" of Bordet is prepared by heating ox serum to 56° for thirty minutes; the amboceptor is then removed by the addition of about twice the amount of corpuscles which could be sensitised by the amount of serum taken. The corpuscles should be centrifugalised and the supernatant saline pipetted off before adding the serum. The serum and corpuscles are incubated for one hour and centrifuged. The supernatant fluid is "ox colloid."

The absence of amboceptor was tested in each case by the following experiments:—

Guinea-pig cells, 0·2 c.c. + 0·2 c.c. ox serum 56°
+ 0·2 c.c. guinea-pig complement . . . = hæmolysis.
Guinea-pig cells, 0·2 c.c. + 0·2 c.c. ox colloid +
0·2 c.c. guinea-pig complement . . . = no hæmolysis.

RESULTS.

The *presence of complement* in fresh milk is easily demonstrated by adding to milk red blood cells of the guinea-pig, together with ox serum heated to 56°.

The following is a typical one out of a large number of almost precisely identical series:—

	Red Blood Cells. Guinea-pig. 10 per cent.	Milk.	Ox Serum 56°	Result— Extent of Hæmolysis.
1 . .	0·2 c.c.	0·5 c.c.	0·2 c.c.	Hardly any.
2 . .	0·2 ,,	1·0 ,,	0·2 ,,	Very definite.
3 . .	0·2 ,,	1·5 ,,	0·2 ,,	Nearly complete.
4 . .	0·2 ,,	2·0 ,,	0·2 ,,	Complete.
5 . .	0·2 ,,	2·0 ,, (boiled)	0·2 ,,	None.

Control series without the ox serum showed no hæmolysis, but the cream carried many of the corpuscles up to the top, giving rise to a bright red plug, which is not diminished by centrifuging, and which will be discussed later. Better results are obtained with centrifugalised milk.

The presence of complement in the whey can be demonstrated by a precisely similar series. Only on one occasion did I fail to find complement in the milk,—that was in the case of a cow that had calved on the previous day.

The Presence of Amboceptor.—Red corpuscles added to milk are not hæmolysed,[1] but after the addition of the "ox colloid" the following series was constantly obtained:—

	Red Blood Cells. Guinea-pig. 10 per cent.	Milk.	Ox Colloid.	Ox Serum. 56°	Result as to Hæmolysis.
1 . .	0·2 c.c.	2·0 c.c.	...	...	None.
2 . .	0·2 ,,	2·0 ,,	0·2 c.c.	...	Nearly complete.
3 . .	0·2 ,,	2·0 ,,	...	0·2 c.c.	Nearly complete.
		Whey			
4 . .	0 2 ,,	2·0 ,,	...	...	None.
5 . .	0·2 ,,	2·0 ,,	0·2	...	Nearly complete.
6 . .	0·2 ,,	2·0 ,,	...	0·2 ,,	Nearly complete.

Thus the same results were obtained with both ox colloid and ox serum, therefore there must be amboceptor present in milk.

Both amboceptor and complement appear to be present in about one-tenth the amount of that in the serum, but vary slightly from day to day.

Agglutination.—The *red plug* which is found when guinea-pig corpuscles and milk are mixed has been already referred to. If the formation is watched under the microscope by oblique illumination a rapid agglutination is seen, which also involves the fat globules, which accounts for the fact already stated, that on centrifuging, the corpuscles are carried to the top with the cream. The agglutination is complete in about twenty to thirty minutes, and does not occur if centrifugalised milk is used. The merest indication of agglutination takes place if goat cells be used, and none at all with ox cells. Goat cells are not hæmolysed by ox serum, and do not give the red plug; the cream rising after centrifuging may have a very faint pink tinge. The plug is quite white when ox cells are used. The plug is therefore formed by the agglutination of the corpuscles with cream, and appears to be dependent for its production upon the presence of hæmolytic factors, when the conditions are not such as to give hæmolysis.

It fails to occur after heating the milk to 64° for half an hour, or by heating to 70° or upwards for a few minutes. It is obtained with milk bought at a shop. These facts show that mere traces of complement are sufficient to produce it, since it occurs when there is not sufficient complement to produce hæmolysis, but does not occur when no potential hæmolytic system is present. Also it is not destroyed at 56° C.—at a temperature when so much of the complement is destroyed that no hæmolysis can be obtained. If the hæmolysis in the tube is very strong the red plug is not found, the cream at the top being merely tinged or even white.

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[1] On one occasion some hæmolysis was obtained with milk and corpuscles only,—in a cow who had recently calved, about five days after parturition.

Its absence in milk which has been boiled or kept a few minutes at 70° C. makes its formation available as a test for milk which has been pasteurised in the usual way, where the temperature is above 80° C.

SUMMARY.

1. Milk when fresh contains both complement and amboceptor in about one-tenth strength that of serum.

2. In order to obtain hæmolysis, "ox colloid" must be added, thus confirming Bordet's observations.

3. On adding blood cells, which are capable of being hæmolysed by fresh ox serum to milk, the cream picks up the corpuscles and carries them to the top, forming a bright red plug. This plug is absent with milk heated for a few minutes to 70° C., and may be used as a test for heated or pasteurised milk.

I have much pleasure in taking this opportunity of thanking Dr. Dean for his invaluable assistance and advice during the course of these experiments.

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37.

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On changes in the amount of higher fatty acids to be obtained from the liver after removal from the body.

By

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In a short note published in the Proceedings of the Physiological Society in February 1904, an account was given of the results of some experiments in which it was found that the amount of higher fatty acids present in various combinations in the liver increased when the liver pulp was kept in flasks at body temperature in the presence of antiseptics with a current of moist air sent through them, whereas if samples of the same liver pulp were heated in a boiling water bath before incubation, the change did not occur.

Since then a number of other experiments have been carried out on the same lines and as before in a certain number of these the same sort of increase has been observed. The results of these experiments have not hitherto been published, because though when the increase is observed it is often very marked, it is nevertheless very frequently not found, and as it has not yet been found possible to determine what the conditions are on which it depends, the further study of the significance of the phenomenon is rendered exceedingly difficult. Since however when there is no increase there is no diminution, or almost without exception at most an insignificant one, amounting to less than 5 perc. of the fatty acids present, the phenomenon when it occurs cannot be put down to mere analytical error.

In estimating the interest attaching to such a change it is of course, of the greatest importance that the methods employed should be most carefully checked. This can be done by taking two control samples, in which the fatty acids are determined at the time when the other samples are first put into the thermostat. The difference between the results of these two control estimations marks out the probable range of error in the estimations on the samples in which after incubation a change is looked for.

In the first six experiments when the estimations were carried out by Rosenfeld's method the average error so determined was ± 4,0 perc. of the total amount of extract, the maximum being 5,5 and the minimum 3,2 perc.

As was mentioned in the earlier communication referred to above it was observed that the extract obtained in that way gave on saponification only 60 to 65 perc. of insoluble fatty acids and sometimes less than that: and as the yield of higher fatty acids, which was the point under investigation, did not bear a constant ratio to the yield of extract, in the next series of experiments, the later ones referred to in that note, the extracts obtained by Rosenfeld's method were saponified and the weight of insoluble fatty acids so obtained was determined for the observation of the change. In the ten experiments carried out in this way the average range of error in the two controls was ± 0,9 perc. of the amount of higher fatty acids, the maximum being 2,0 perc. and the minimum 0,1 perc. This method was however cumbersome and in later experiments the estimations were simplified by weighing out the liver pulp for the several samples and then either at once or after incubation for 24 hours or more, heating the whole pulp with strong potash as in Liebermann's method of fat estimation, precipitating the insoluble acids, filtering them off and after drying them on the filter extracting them in a Soxhlet apparatus with petroleum ether. In the seventeen experiments done in this way in which the range of error was determined by two controls, it was found to be, taking the mean of all, ± 1,1 perc. of the amount of fatty acids; only on one occasion was it more than 2 perc., in that case as much as 3,5 perc., and on seven occasions 0,5 perc. or less.

In a recent paper by Paul Saxl 1) three experiments are quoted in which the attempt was made to get some confirmation of the results referred to. In each of these cases two controls were taken giving as the range of error ± 7,7, 11,8, and 10 perc. respectively Now since a change amounting to more than 20 perc. is exceptional, but changes of 10 and 15 perc. common, if the error of estimation is itself 10 perc. such changes when found have little significance. For instance Saxl describes an experiment in which, with two controls showing a ± 10 perc. error, he found in two of his

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1) P. Saxl. Hofmeisters Beiträge, Vol. X, p. 447.

samples an increase of 25 perc. and 35 perc. respectively as compared with the mean of his controls. He proceeds to say that such changes lie within the limits of error; and it may be felt that what he says, though not strictly and verbally true, has some logical force. I have only once found an increase greater than 35 perc., but with the probable error reduced to ± 1 perc. the inference drawn by Saxl loses its logical justification entirely, even when the increase is something less than 35 perc.

As instances of experiments of this kind may be cited the following;

No. 8. Two rabbits livers rubbed to a pulp through fine nickel wire gauze (10 meshes to 1 cm).

The higher fatty acids insoluble in water, soluble in petroleum ether, reckoned for 100 g of the dry weight of liver substance were:

In 2 controls.	In 2 samples kept at 37° C. for 48 hours.	In 2 samples similarly treated but with Phosphorus added.
5.04 4.98 } 5.01 ± 0.6 perc.	(1) 6.28 (2) 6.31 } 6.29 ± 0.2 perc.	(3) 6.31 (4) 6.21 } 6.26 + 0.8 perc.

In the four samples the increase amounted to about 25 perc. of the fatty acids present in the liver: the absolute increase in weight of fatty acids was in (1) 0,14, in (2) 0,08 in (3) 0,08 and in (4) 0,09 g.

No. 10. Pig's liver treated as in No. 8.

The fatty acids reckoned on 100 g dry weight of liver substance were.

In 2 controls	In samples kept at 37° for	In samples heated in boiling water for 5 minutes and then at 37° C. for.
14.58 14.86 } 14.72 ± 1 perc.	(1) 24 hours 16.85 (some loss)	(2) 24 hours 15.33
	(3) 40 hours 18.76	(4) 40 hours 15.53
	(5) 69 hours 19.50	(6) 69 hours 15.72

The increase in the flasks 1, 3 and 5 amounted to 14, 27 and 32 perc. of the fatty acids present in the controls, where the range of error was 1 perc.; and the absolute increase in weight of fatty acids was in (1) 0,278 g in (3) 0,425 and in (5) 0,391 g: in the heated flasks the increase was 4, 5 and 6 perc. respectively.

No. 11. Pig's liver treated as in No. 10.

The higher fatty acids reckoned on 100 g dry weight of liver substance were.

In 2 controls		In samples kept at 37° C.	In samples heated in boiling water bath for 5 mins. then kept at 37° C.
13.49	13.55 ± 0.4 perc.	(1) for 22 hours 16.01 perc.	(2) 14.52 perc.
13.61		(3) for 48 hours 16.89 perc.	(4) 14.83 perc.

The increase in flasks (1) and (3) was 18 and 24 perc. respectively or in absolute weight 0,245 and 0,260 g; in the flasks that were heated before being put in the thermostat 7 and 9 perc.

No. 22. Pig's liver treated as before.

The fatty acids in 100 g of the fresh pulped liver substance were.

In 2 controls		In 2 samples kept at 37° C. for 48 hours		In 1 sample to which 2 c. c. bile was added then kept at 37° C. for 48 hours
2.78	2.81 ± 1 perc.	(1) 3.16	3.22 ± 2 perc.	(3) 3.54 perc.
2.84		(2) 3.29		

In the flasks to which nothing was added the increase was about 15 perc. and the absolute gain in weight of fatty acids was 0,146 and 0,252 g respectively. In the flask with bile added the gain was 26 perc. or 0,229 g. The absolute error in the controls compared with the mean was — 12 and + 24 mg respectively.

No. 31. Dog's liver treated similarly.

The fatty acids found in 100 g of the fresh pulped liver substance were.

In 2 controls		In one sample kept at 37° C. for 40 hours with stream of air	In two samples at 37° C with stream of hydrogen
3.03	= 3.03 perc.	(1) 4.00 perc.	(2) 2.94 perc.
3.03			(3) 2.95 perc.

In flask (1) the increase amounted to 32 perc. and the absolute amount gained was 0,287 g. In the flasks through which hydrogen was passed instead of air the loss amounted to about 3 perc.

No. 39. Dog's liver removed aseptically and minced in a sterilised mincing machine into sterilised flasks.

The fatty acids found in 100 g of the fresh minced liver substance were.

In 2 controls		In the flask kept at 37° C. for 40 hours
2.31	2.33 ± 0.8 perc.	3.09
2.35		

The increase in this case amounted to 32 perc. and in actual weight to 0,240 g. Broth tubes inoculated from the flasks at the end of the experiment were absolutely clear after 48 hours incubation at 37° C.

No. 38. Dog's liver removed with aseptic precautions minced in a sterilized mincing machine of special pattern into sterilized flasks.

The fatty acids found in 100 g of the fresh minced liver were:

In 2 controls		In flask kept at 37° C. for 40 hours
7.64	7.78 ± 2 perc.	9.53
7.91		

The amount of fatty acids found here was 22 perc. more than that found in the controls, the increase amounting to 0,58 g. Broth tubes inoculated from the flask at the end of the experiment showed that infection had occurred.

In all these cases the increase in the amount of fatty acids found after incubation was from 10 to 40 times as great as the amount by which the two controls differed from the mean between them. This seems to make the significance of the change noted, different from that in Saxl's experiment even though the percentage increase was in his experiments on one occasion 35 perc. and in these cases never as much as that.

The significance of changes of this kind may be gauged in another way. An increase such as that indicated in the experiment of Saxl's referred to above, when quoted as amounting to 35 perc., sounds considerable; but when it is noted that it is after all only a matter of 35 mg a different impression is obtained and the conclusion to which Saxl himself comes, appears inevitable, especially when the range of error as determined by the two controls is taken into account at the same time. If however, the absolute difference of weight as compared with the amount pointed to by the control estimations is ten times or more than ten times as much as this, the aspect of the case is changed. And conversely an increase expressed as so much per cent. of the total fatty acids present in the controls may be small but the absolute weighed difference may be such as to give a significance to the small percentage, which it by itself would not convey. Thus in one experiment (Nr. 24) on the pulp from a sheep's liver, two weighed portions of the fresh pulp contained more than 5 g and 4 g of higher insoluble fatty acids, forming 12,33 and 12,18 perc. of the pulp, respectively, in the one case 30 mg more and in the other 23 mg less than the mean percentage of the two, the mean error being ± 0,5 perc. of the amount of fatty acids present. In five portions of the same pulp kept at 37° C for a period of 40 hours the increase found, reckoned per 100 g of the fatty acids present, was in four of them not more than 5 perc. and in the fifth only 6 perc. This, it is true, is an increase ten times greater than the error revealed by the controls in this experiment, but expressed in percentage looks small. The absolute amount of fatty acids, which this small percentage increase represents, is however not insignificant (0,382, 0,258, 0,258, 0,179 and 0,310 g) amounting in four of the five to a quarter of a gramme or more. In most of the experiments quoted above the absolute weight by which the fatty acids were found to have increased, was of the same order as this and in one case it was more than half a gram. The actual weights are given at the end of the protocols.

On these grounds I think that though Saxl may be justified in arguing that the change he noted falls within the limits of experimental error, the changes in the experiments here recorded lie well outside those limits.

It is true that these experiments are selected from a large number, many of which did not show the change which in the selected cases, as well as several others, was obvious. Of these negative experiments there were however none that exhibited changes in the opposite sense, a diminution of the fatty acids, in any way comparable to the increase observed in the positive experiments. Generally in each experiment there were half a dozen and sometimes more samples put into the thermostat. Now and again one determination in a series would show a diminution, about half a dozen out of some hundreds of more than 5 perc., one as much as 12 perc. But in almost all such cases there were obvious and satisfactory technical reasons for expecting a low figure, while the great majority of the negative experiments tend to confirm the validity of the positive ones, inasmuch as the figures in the former show throughout the series only trifling variations from the controls The following instances may be cited:

No. 9. 2 rabbit's livers reduced to pulp by rubbing through wire gauze.

The fatty acids reckoned for 100 g dry weight of liver substance were.

In 2 controls, one with Phosphorus added, the other with none	In 3 flasks kept for 10, 32, and 69 hours at 37° C.	In 3 flasks, to which phosphorus was added, kept for the same times at 37° C.
6.51	6.41	6.47
6.45 ± 0.8 perc.	6.63	6.45
6.40	6.47	6.47

No. 21. Pig's liver.

The higher fatty acids reckoned for 100 g of fresh liver pulp were:

In 2 controls		In 2 flasks kept at 37° C. for 44 hours	In 4 flasks to which certain substances were added experimentally.	
4.57	4.53 ± 0.9 perc.	4.64	4.73	4.76
4.49		4.46	4.79	4.84

Small increases up to 5 perc. were found in some of the samples (amounting to in one case 0,118 g) apparently due to the additions; reference will be made to these later.

No. 23. Sheep's liver; the fatty acids reckoned on 100 g fresh liver substance were:

In 2 controls		In 2 flasks kept at 37° C. for 24 and 48 hours	In 6 flasks with various substances added as in Nr. 21		
5.07	4.89 ± 3.5 perc.	(1) 5.14	(3) 4.94	(5) 4.89	(7) 5.41
4.71		(2) 4.78	(4) 5.09	(6) 4.77	(8) 4.79

The only sample in which there was any marked change was No. 7, showing an increase of 10 perc. or 0,32 g; the substance added being 2 cm. of the animals own bile.

No. 36. Pig's liver; the fatty acids in 100 g of fresh liver substance were:

In 2 controls	In 2 flasks kept at 37° C. for 40 hours	In 2 flasks with certain substances added kept similarly
4.04 perc.	3.95	4.00
4.03	3.88	3.99

It is such results as these, occurring fairly frequently, that have made the study of the conditions upon which the positive results depend difficult to pursue. But they serve nevertheless to confirm the belief that the positive results, when they do occur, are not due to error or accident.

The possibility that the change observed was the work of micro-organisms and not due to surviving reactions among the components of the liver cells, as mentioned in the earlier note, was dealt with, by taking cultures in broth of micro-organisms present in samples, in which the fatty acids were found to have increased, and adding them to boiled samples in another experiment: neither these samples nor those which were not boiled and to which no culture was added showed any change. It is true that the antiseptic measures taken in the great majority of the experiments though they always prevented putrefaction, did not whenever tested, destroy micro-organisms entirely. But in three experiments in which the liver was excised in the laboratory under aseptic precautions no growth was obtained at the end of the experiment on inoculating tubes from the flasks. In two of these three experiments, there was found to have been an increase in the fatty acids, in the third none. One is quoted above on p. 330 (No. 39). The others were as follows:

No. 37. Rabbit's liver minced in sterilized mincing machine.

Control flask	Flask kept at 37 ° C. for 40 hours
2.77 perc.	3.28 perc.

The increase amounted to 18 perc. or 0,125 g and this in spite of the fact that the flask in which it was observed was the first to be charged by the machine, and received the bile and blood, which this form of mincer delivers first before any pulped liver tissue comes through, which bile and blood was therefore weighed as liver substance, though it would have yielded unweighable traces of fatty acids.

No. 41. Dog's liver.

Control	Flask kept at 37 ° C. for 40 hours
2.83 perc.	2.85 perc.

In this case the flask to be first filled, which received the blood and bile expressed from the liver was, not kept.

In another experiment done in the same way infection took place during the excision of the liver, as in No. 38 quoted above (p. 331) but here too the flask incubated showed some increase.

No. 40. Dog's liver.

2 controls	2 flasks kept at 37 ° C. for 66 hours	
2.94	(1) The first to be filled	2.98
2.94	(2)	3.17

In flask (2) the increase amounted to 8 perc. (0,14 g). But both flasks had been infected in removing the liver.

Thus the change was observed in two out of three cases in which no micro-organisms were found to be present, and is therefore not the work of micro-organisms. Except in their own bodies micro-organisms are not known to produce higher fatty acids, so that there is a priori no probability of the change being the work of bacteria. On the other hand the fact that in one case, in which the material was found to have remained sterile, the change was not to be observed, would appear to indicate that the change when absent is not absent because of the interference of bacteria or their products with the reaction to which the change is due, unless it be possibly in certain cases certain particular species of bacteria. Rather, the facts suggest that in certain phases of hepatic activity the conditions exist for the synthesis of higher fatty acids, in certain other phases they do not, and an increase in the amount of fatty acids is found when the material used is obtained in the one set of conditions and not when it is in the other.

A large number of experiments were done in which various substances were added to see if they influenced the result either by supplying material for the synthesis, or by determining the development of conditions favourable for it to occur. But the results were not sufficiently constant to allow any inference to be drawn with certainty. In the earlier experiments on more than one occasion the addition of glycogen seemed to favour the change: but as the result of subsequent experience this cannot be said to be a constant result. Glucose was also tried and a number of substances which might hypothetically be supposed to be intermediate products in the transformation of carbohydrate to fat. But in these cases too, though sometimes the samples to which the additions were made showed a more marked change than those to which nothing was added, on other occasions there was no difference. Similarly the addition of small quantities of bile or an extract of pancreas, prepared as Cohnheim has described in his experiments on muscular glycolysis, has in certain cases appeared to exert a marked influence, but not in all. The addition of phosphorus or arsenite of soda also was without effect. (Experiments No. 8 and 9 sup. pp. 329, 332.)

The results therefore must for the present be merely recorded, without any pretention to throwing light on the nature of the processes involved or the conditions under which they are carried out. It may be that the inconclusive experimental data not detailed in this or the previous account may yet in the light of later experience be of some service.

Somewhat similar results were described by Hahn[1]) who found an increase in the fatty acids obtainable from compounds in the blood, when this was collected aseptically and kept for a time at the body temperature and that the increase was more marked if sugar was added. On the other hand in additioen to those published by Saxl, four negative results with the liver were described by Siegert 1), who removed the liver of dogs aseptically and allowed pieces to undergo autolysis at the body temperature, estimating the fatty acids by extraction with alcohol and ether and saponification of the extract.

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[1]) Hahn. Münchener Medizinische Wochenschr. April, 19, 1904.

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1) Siegert. Hofmeisters Beiträge, Vol. I, p. 114, 1902.

N 17

A Lecture

ON THE

FUNCTIONS OF THE LIVER IN RELATION TO THE METABOLISM OF FATS

Delivered before the Harvey Society of New York on
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BY

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A Lecture

ON

THE FUNCTIONS OF THE LIVER IN RELATION TO THE METABOLISM OF FATS.

GENTLEMEN,—The liver is an organ with multifarious duties to perform. Of many of these it is possible to speak with some degree of scientific assurance. Something of its work in connexion with carbohydrate storage has been known for 60 years. It has something to do with the removal of nitrogen from superfluous protein foods and with the manufacture of urea from this nitrogen. It takes its part in the transformation of purin bases, turning them out as uric acid or destroying them more completely still. It deals with the blood pigment that is past work and uses it to prepare colouring matter for the bile, the urine, and the fæces, and there are many other miscellanies in the chemistry of the body which have been put down to its activity.

But the fact that the fat absorbed from the food is specially diverted, unlike the other foodstuffs, from the stream of portal blood and conveyed by its own channels into the systemic circulation might be taken as an indication that the fats, at any rate, may be left for other organs and tissues to deal with. Fats are stored in the body in larger quantities than any other kind of food. But the storage places for fats are well known and the liver in mammals is not included among them.

Yet the examination of the amount of fat in the livers of men and other animals reveals very great variations, not only in the case of livers from the post-mortem rooms attached to hospitals for the sick, but in those removed from animals at the slaughter-house. A hard-and-fast distinction between these two classes of material it may not be possible to maintain, but anyone who has determined the amount of fat in the liver in a long series of animals that are presumably normal will have been struck by figures occasionally obtained that are very different from those that may be taken as normal average figures for this organ, different even from those that would be abnormally high for other organs. Taking one series of about 40 animals, the livers of which I examined in this way, the great majority were found to yield between 2 and 4 per cent. of the higher fatty acids reckoned on the fresh weight of the tissue as the total amount present in all the different combinations of fatty acids the liver contains, but five out of the series, or 1 in 8, gave on analysis double or more than double this amount: a dog 7·8 per cent., a pig 8·3 per cent., and three sheep 7·3, 12·3, and 14·1 per cent. respectively. This last figure means that very nearly, if not quite, half the solid matter of the tissue was fat in some form or other. The same fact comes out in spite of the absence of extreme figures in some analyses carried out by Hartley at the Lister Institute. A series of nine analyses of the livers of guinea-pigs which had been fed on oats, cabbage, and hay showed the percentage of higher fatty acids reckoned on dry weight to range from 6·1 to 14·9. Another series of five analyses on guinea-pigs fed exclusively from the age of two weeks on bread, water, and cabbage gave variations from 6·7 to 12·6 per cent. A series of 12 rats that I examined gave a range of variation from 1·7 to 6·5 per cent. of the fresh tissue. In another series of rats examined in the same laboratory by the same methods there were 14 the livers of which contained less than 4·2 per cent. of higher fatty acids, the mean percentage being 3·1; while four others with more than 4·2 per cent. gave as the mean 7·2 per cent. I shall be referring later to some figures which I obtained from cats; five of these animals, to all appearance normal, gave the figures 2·2, 3·5, 3·9, 19·2, and 22·2 per cent. From the post-mortem room material may not infrequently be obtained in which still larger amounts than these are to be found; 60 or 70 per cent. of the dry tissue, and 23 per cent. or more of the fresh, may be isolated as higher fatty acids after treatment with potash and alcohol and subsequent acidification.

A good deal of experimental work directed to the production of abnormal amount of fat in the liver and other organs has been published by G. Rosenfeld, whose results are well known.[1] The method of estimating fat employed by him is one that is open to a number of criticisms. On the one hand, it must be admitted that it yields a larger weight of substance soluble in ether than other methods. But, on the other hand, it is impossible to compare the results obtained by this method even with one another, since there are many combinations of fatty acids which are soluble in ether: the free acids themselves, the neutral glycerides, the choline phosphoric acid combinations of the type of lecithine, jecorine, cuorine, and others as well. An ethereal solution may in one case be composed almost entirely of neutral glycerides; in another case more than half of it may be some phosphatide lipoid substance. Since the common component of all these compounds which is of greatest significance is the fatty acid radical, the most satisfactory method of estimating fats for most comparative purposes is to set the fatty acids free from all the combinations in which they occur, separate them from the other constituents, and weigh them. If the combinations from which they are obtained are simple neutral fats the yield will be about 95 or 96 per cent. of the fat. If they are lecithin it should be 70 per cent., whereas other phosphatides yield less than 50 per cent. The fat contained in the ethereal solutions of extracts of the heart or liver obtained by Rosenfeld's method do not commonly yield more than 60 per cent. of fatty acids, sometimes less, and this yield varies according to the composition of the extract. Nevertheless, the range of variations in the amount of the fat in the liver that Rosenfeld was able to produce experimentally was so much greater than in the case of other organs that such uncertainty as his method leaves is relatively insignificant. There is no organ that is ever flooded with fat in the way that the liver is.

Now the appearance of large amounts of a substance in an organ may be due to active production of that substance in that organ or else to importation from other parts. We know from the famous experiments of Rothamsted, carried out by Lawes and Gilbert 60 years ago, that fats are synthesised by animals and that the material used for this synthesis is in the main at least the carbohydrates contained in our food. Magnus Levy has described a reaction occurring in the liver which he regards as typical of the unknown reactions by which this synthesis is effected.[2] He found among the acids formed in the liver when portions of this organ were kept aseptically in a thermostat for some days, not only acetic and lactic acid, but also butyric acid, and at the same time gas was given off which proved to consist in part of hydrogen. The source of the butyric acid could not be determined positively, but there were some indications that it was derived from sugar and the simultaneous occurrence of hydrogen suggested an analogy with the butyric fermentation of sugar. And Magnus Levy adopts as the explanation of the appearance of this butyric acid a hypothesis originally put forward by Nencki, which may be extended to account for the formation of higher fatty acids from carbohydrate. This is, that in the first instance lactic acid is formed from the sugar; that lactic acid, as is well known to be the case with all α-hydroxy acids,[3] readily splits into formic acid and the aldehyde with one less carbon atom than the hydroxy acid, in the case of lactic acid acetic aldehyde; that the aldehyde then condenses to form aldol, which is the β-oxybutyric aldehyde, from which it is easily conceivable that butyric acid may be formed. The condensation of aldehydes on this plan is not confined to acetic aldehyde, and either by the condensation of two molecules of aldol or four of acetic aldehyde it is equally conceivable that the same type of change as leads to the formation of butyric acid may lead to the formation of caprylic acid with eight carbon atoms, and so forth. The only limitation such a hypothesis imposes is that the acids produced must all contain some multiple of two carbon atoms, which is just what is well known to be the case with the fatty acids found in nature. If, therefore, butyric acid is formed from glucose in the liver by such reactions as these the synthesis of palmitic and oleic and stearic acids from glucose may also be the work of the liver and be effected by similar reactions. In butyric fermentation of sugar, besides butyric acid sufficient of the six-carbon caproic acids is produced to be commercially worth separating, and as Raper showed at the Lister Institute detectable and identifiable amounts also of the normal eight-carbon caprylic acid.[4] The reactions, therefore, which serve the bacteria for producing butyric acid from sugar serve them also for the synthesis of caproic acid and caprylic acid. Raper also succeeded in synthesising normal caprylic acid from acetic aldehyde by

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[1] Archiv für Experimentelle Pathologie und Pharmakologie, vol. lv., pp. 180 and 350.

[2] Hofmeister's Beiträge, vol. ii., p. 261.

[3] Journal of the Chemical Society, 1904, p. 1708

[4] Journal of Physiology, vol. xxxv., p. 24.

condensation of aldol with itself, oxidising the aldehyde so formed by means of silver oxide and reducing the hydroxyl groups in the chain with hydriodic acid,[5] an achievement which previous chemical experience as to the condensation of aldehydes resulting in the formation of branched and not of normal chains rendered improbable. The presence of hydroxyl groups in the chain seems to account for this. Magnus Levy's observations, therefore, may be taken as pointing to the liver as a likely organ to which to assign the transformation of sugar into fat, since it has to be assigned to some organ in the body.

In a long series of experiments on the amount of higher fatty acids present in the liver I found that in a fair proportion of cases, nearly 50 per cent., this amount could be observed to increase when a pulp of the liver tissue was kept in a thermostat at the body temperature.[6] The weighed increase of fatty acids soluble in petroleum and not soluble in water from a sample of liver pulp weighing about 50 grammes was sometimes as much as 3, 4, or even 5 decigrammes and amounted to a 15 to 30 per cent. increase on the fatty acids present in the pulp before the experiment started, as determined by reference to the mean between two control estimations, which differed from that mean by about 1 per cent. of the total amount of the fatty acids present. In three out of five attempts to remove and reduce the liver to pulp aseptically this was successful, and in two of these three there was a marked increase in fatty acids on incubation, so that the formation of fatty acids was not the work of bacteria. As to the nature of the material which was converted into fatty acids under the conditions of the experiments, it was impossible to get clear evidence: the addition of substances which might be supposed to be used, or to occur in the course of the reactions involved, in no case gave constant and uniform results. The many negative experiments seemed to make it desirable to postpone further investigation till more light from other sources could be thrown upon the subject.

There are, therefore, grounds for looking to the liver as the seat of the synthesis of fatty acids, and it is possible that the reason why there are greater variations in the amount of fat in the liver than in other organs is in some measure to be sought for in this direction. It should be remembered, too, that the fatty acids found in the liver are to a much greater extent than is the case in the connective tissue fat, unsaturated acids ; and since the only hypothesis as to how fatty acids are synthesised for which there is anything to be said implies a possibility or even the probability that the acids formed would be unsaturated, this may be regarded as some further justification for the idea.

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[5] Journal of the Chemical Society, 1907, p. 1831.

[6] Schmiedeberg's Festschrift, 1908.

There are, however, still better grounds for thinking that large amounts of fat found in the liver are often due to the importation of fat from the storage places in the connective tissues. For in the first place, perhaps the most remarkable feature of the extreme cases of fatty liver described by Rosenfeld in dogs which were starved for five days and then given phlorrhizine on the sixth and seventh days, is the extraordinary rapidity with which enormous quantities of fat appear in the liver, forming as much as 70 per cent. of the dry weight of the organ.[7] He says that the fat does not begin to accumulate until the fortieth hour after the first dose of phlorrhizine, and the animal is killed eight hours later. According to this account, therefore, this enormous increase in the amount of fat in the liver takes place in the course of some eight hours. Again, if the animal is allowed to live another 24 hours, without any further dose of phlorrhizine the liver is then found to contain only normal amounts of fat, so that recovery is nearly as remarkably rapid as the development of this abnormal distribution of the fat in the body is. It would be easier to understand such rapid changes as these if it is not necessary to suppose the fat to have been made where it is found.

There are probably parallels to be found to the rapid changes indicated by Rosenfeld's results in clinical experience. Cases are recorded of the most intense fatty changes in the liver being found post mortem when there is every reason to suppose that the abnormal condition has come on with the greatest rapidity. Cases of deferred chloroform poisoning particularly suggest themselves. Thus Langmead[8] describes the case of a boy, aged 12 years, who after operation under chloroform for lymphadenomatous glands in the neck became delirious and later drowsy and unconscious, and died, jaundiced and wasted, 72 hours after the operation with a

[7] Ergebnisse der Physiologie, 1902.

[8] Brit. Med. Jour., Sept. 28th, 1907.

very fatty liver; and another case of an apparently healthy child, aged three and a half years, dying 36 hours after a similar operation with similar sequelæ, and showing intense fatty change in the liver. He also describes a case where similar clinical symptoms followed a trifling operation under gas in an out-patient, a girl, aged 15 years, accompanied as in the other cases by alarmingly rapid wasting. This case ended, however, in recovery, and ten days after the onset the body was restored to its normal condition of nutrition. Cases of cyclical vomiting ending fatally in three or four days are described with similar post-mortem appearances in the liver.

A case of a peculiar character has recently come to my notice through the kindness of Dr. A. E. Boycott, who sent me a portion of the liver for examination. A man, aged 49 years, apparently healthy, drank when intoxicated a considerable amount of strong hydrochloric acid and died six hours later. His stomach was practically destroyed. An adjacent portion of the liver was also burnt and this part under the microscope showed no more fat than is often to be seen in a normal liver. The rest was intensely fatty and contained on analysis more than 20 per cent. of higher fatty acids reckoned on the fresh weight. The acids had the iodine value 65·6. Dr. Boycott's comment that "the presumption is pretty well justified that most of this fat had appeared in the liver in these six hours" is one with which I am strongly disposed to agree. Those who have a wider clinical and post-mortem room experience than I, may be able to furnish further evidence for the belief that I hold that the most marked accumulations of fat in the liver are not necessarily by any means always slowly brought about by chronic disorders as in phthisis or chronic alcoholism. In many cases they may have arisen as rapidly, and in others perhaps are recovered from as rapidly as seems to be the case in phlorrhizine poisoning in dogs.

It is unfortunate that the pathological examination of post-mortem room material is so largely confined to the histological and microscopic appearances. The estimation of the amount of fat in a tissue by histological reactions is a well-known source of fallacy. Hoppe Seyler showed that there is less fat in a sciatic nerve that has degenerated as a result of section than there is in the healthy nerve on the other side of the same animal.[9] Rosenfeld proved the same to hold for a degenerated occipital lobe in man by comparison with the healthy lobe on the other side of the same brain. It is also familiar that whereas normal heart muscle gives no histological reaction for fat, it nevertheless contains something like 10 per cent., reckoned on dry weight, of higher fatty acids in different kinds of combinations, of which the microscope gives no indication. The hearts of animals, on the other hand, that give histologically the most intense scharlach reactions for fat, on analysis may be found to contain relatively only a little more fat than normal hearts.[10] The chemical estimation of fat in tissues, when all appliances are at hand, hardly takes more time than the preparation of histological specimens, and it is urgently desirable that post-mortem room material should be systematically worked at on chemical lines as well as histological.

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[9] Virchow's Archiv, vol. viii., p. 127, and vol. xx., p. 426.

The classical controversy as to the source of the fat in fatty organs is, as a matter of fact, commonly regarded as closed. Lebedeff 25 years ago made the two most important observations bearing on this point, and they have been confirmed by Rosenfeld using other methods. Lebedeff, in the first place, noted that the liver in a case of phosphorus poisoning supervening on extreme emaciation showed no fatty change. He suggested that the absence of the usual amount of fat found in the liver in phosphorus poisoning was due to the lack of fat in the rest of the body.[11] Rosenfeld has since administered phosphorus to dogs in different states of nutrition. He found in the liver of a well-nourished dog 37 per cent. of fat reckoned on dry weight; in a dog less well stocked with fat 17·4 per cent.; and in a dog reduced by starvation to extreme emaciation only 6·1 per cent., a low figure, that is to say, even for the liver of a normal animal.[12] Lebedeff's other observation was that if he substituted for the fat usually present in a dog's adipose tissue fat of another kind, linseed oil, the acids constituting which are liquid, and give lead soaps that dissolve in ether, then the administration of phosphorus causes the appearance of large amounts of fat in the liver similar in composition to the linseed oil contained in its adipose tissue, a fat, that is, yielding 67 per cent. of acids the lead soaps of which dissolve in ether, whereas a dog not previously prepared when given phosphorus had in the fat in its liver only 23 per cent. of such acids giving soluble lead soaps. By means of the iodine value Rosenfeld has, as is well known, been able to show that the fat found in such large quantities in the liver in phlorrhizine poisoning varies in its character with the character of the fat stocked in the animal's adipose tissue.

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[10] Problems in Animal Metabolism, p. 93 (J. Murray, 1906).

[11] Pfluger's Archiv, vol. xxxi., p. 11.

[12] Zeitschrift fur Klinische Medicin, vol. xxxvi., p. 232.

The most marked changes, therefore, in the amount of fat in the liver are due apparently to the migration of fat from the adipose tissues in which it is stored. For if it were produced in the affected organ there is no reason why the character of fat produced should be determined by the nature of fat in other parts of the body. And, as we have seen, the liver is the organ in which the most marked accumulations of fat are found. The liver is therefore the organ to which the migration of fat from other parts of the body is most conspicuous. In other organs the amount of fat may be increased 30 or 50, possibly 100 per cent. above normal mean figures. In the liver the increase may be 300 or 400 per cent. above the normal mean, and even among normal animals something like 1 in 10, or even 5, give figures that are 50 to 100 per cent. or even more above those which prevail among all the rest. In fact there is in the extent and range of the variations in the amount of fat in the liver, as compared with other organs, something comparable to the range of variations in the amount of glycogen in this organ compared with others. But the interpretation of the appearance in the liver of the largest amount of fat ever found there so far seems to be different, in that the fat is in the cases that have been most studied imported from other parts of the body and not made on the spot.

Now there are important differences between the fat usually found in the liver or heart and that of adipose tissue. Substances soluble in ether extracted from the heart or liver by Rosenfeld's method are not entirely composed of fat in the ordinary sense of neutral glycerides of higher fatty acids: the yield of these acids on saponification is 30 to 40 per cent. too low. It is too low even for lecithin which is known to be present in these organs, and since some neutral glyceride is there too, there must be also some substances present, and not in negligible amounts, besides the simple glycerides and lecithin, either substances which contain less higher fatty acids than lecithin, such as jecorin or cuorine, or else others which contain none at all, such as cholesterin.

After having used, therefore, the Rosenfeld method of fat estimation for a time I made it a practice to check it by saponifying the extracts obtained and then determining the fatty acids. It is the fatty acids after all that are the essential factors in the metabolism of fat, and therefore for general biological purposes some method by which all the fatty acids, present in a tissue in whatever kind of combination, are set free and obtained in a weighable form is the most suitable and reliable. For some years now we have used some modification of the Liebermann method consisting essentially in dissolving the tissue in strong potash and saponifying in the presence of alcohol all compounds of fatty acids, precipitating the acids with sulphuric acid, and extracting with petroleum boiling under 70° C. This method gives excellent results for most purposes. But it soon became obvious that the fatty acids obtained from the liver or heart were different from those in the fat of adipose tissue. When kept for a few days at the ordinary temperature, not protected from the air, or when heated for an hour or two, they increase in weight and are found to have become incompletely soluble in petroleum. Since the oxy-acids formed by oxidation of unsaturated acids are insoluble in petroleum this suggested an explanation of the behaviour of the acids. And the fact that Rosenfeld's determinations of the power of absorbing iodine possessed by the extracts obtained from the liver and heart gave figures higher than those carried out on connective tissue fat, in spite of the fact that they contained only about 60 per cent. of fatty acid, though it might be explained in other ways, might probably mean that the acids in the fatty substances he obtained were more unsaturated than those found in the adipose tissue. If the absorption of iodine by the extract was entirely due to unsaturated fatty acids, then in an extract absorbing 70 per cent. of iodine and containing only 60 per cent. of fatty acids, the fatty acids themselves must absorb about 116 per cent. of their weight of iodine. When Hartley therefore undertook a systematic investigation of the nature of the fatty acids in the liver and other organs this was the first point he attacked. By the use of methods calculated to expose the acids as little as possible to the risk of oxidation he found that the mixed fatty acids of the liver, heart, or kidneys from every species of animal examined absorbed far more iodine than the acids from the adipose tissue fat of the same animals treated in exactly the same way. The iodine value of the fatty acids in these organs averages normally about 120, and ranges from about 110 to 140, whereas that of the acids from adipose tissue varies according to the species from 40 in the goat to 65 in man.[13]

But the one organ, and this is the point that is of most importance in our argument, the one organ in which the iodine value of the fatty acids was found to present fairly commonly wide departures from this order of magnitude is the liver. In the following series of human livers examined by Hartley and Mavrogordato the more the amount of fat exceeds the normal average amount the lower the iodine value of the acids becomes, till in the cases of extreme fatty change the iodine value is no higher than that of the acids in subcutaneous fat. The list given is from their paper.[14]

TABLE I.

Cause of death.		Higher fatty acids per cent. of dry weight.	Iodine value of fatty acids.
Normal figures.	1. Pernicious anæmia	12·1	116·8
	2. Lobar pneumonia	13·7	116·8
	3. Pernicious anæmia	14·25	116·0
	4. Diabetes	14·4	119·6
Commencing fatty change.	5. Toxæmic jaundice	15·6	109·5
	6. Accident	17·2	103·5
	7. Empyema	21·5	96·0
	8. Phthisis	25·4	96·4
	9. Broncho-pneumonia	38·4	84·9
	10. Appendicitis	44·0	91·1
	11. Carcinoma of bladder	47·2	77·8
	12. Broncho-pneumonia	54·6	71·8
	13. Ulcerative colitis	60·9	80·3
	14. Accident	66·3	63·0
	15. Dysentery	73·5	69·1

This is a striking series, but it could be extended by a number of analyses carried out since, many of them on animals, which fully confirm the fact which it brings out—namely, that the more fat there is in the liver the nearer this fat approaches in character to that of the fat stored in adipose tissue. It is difficult to see how this is to be explained except by supposing that when there is an excessive amount of fat in the liver it is because an excessive amount of the stored fat of the body has been brought to the liver. And the inference comes ready to hand that a normal liver, too, gets fat brought to it composed largely of saturated acids but this fat it is able to deal with, converting the saturated acids into less saturated ones. We have not yet obtained sufficient figures for other organs, the heart or kidney for instance, to be able to say positively that variations of this character do not occur there too, but we certainly have not obtained, nor do we any longer expect to obtain, variations on this scale.

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[13] Journal of Physiology, vol. xxxvi., p. 17.

[14] Ibid., vol. xii., p. 371.

The liver, therefore, so far as we can see at present, seems to be the organ of all others to which the connective tissue fat can most certainly be traced. And yet when this importation is not excessive the fatty acids in the liver are like those in the other working organs of the body in having a high iodine value, and in this respect unlike those in the stored fat of the connective tissues. This suggests that the fat which is imported to the liver is normally there altered so as to acquire a higher iodine value. Such a desaturation of fatty acids must occur somewhere, since the fat stored away and not in use is composed of acids that are more saturated than the fat in any of the organs in which we can suppose fat to be oxidised. It may be, of course, that desaturation is effected in all cells which oxidise fat, but the facts with regard to the liver that we have been reviewing make it probable that it is especially in the liver that this change is brought about, and it seems possible that the liver to a great extent prepares the fat in this way for other organs in which the oxidation is subsequently carried to a finish.

In order to see if it was possible to get any evidence of this desaturation of fatty acids in the liver I proposed last summer to Dr. L. Meyer Wedell some experiments in which animals were fed on various fats and oils. Her experiments were unfortunately interrupted, but not before one curious and astonishing fact had come to light. The fatty acids in the livers of rats have the iodine value of about 130, sometimes rather more. Cod-liver oil yielding acids with the iodine value of 150 was given to rats for from 4 to 14 days, and it was found that the iodine value of the fatty acids in their livers went up ; the figures obtained were 161, 198, 170, 173, and 174. Subsequently I repeated these experiments in this way : 5 grammes of ground rice, 4 grammes of plasmon, and 3 grammes of gelatin were heated together in 100 cubic centimetres of water, containing a little sodium chloride, calcium chloride, and potassium phosphate. While still warm this was well shaken with 30 cubic centimetres of cod-liver oil and a fine emulsion obtained. Then while still being shaken it was cooled down under the tap and just before setting poured out into a basin. By determining the amount of this emulsion in jelly that was eaten a rough idea of the amount of oil taken could be formed ; the Tables II. and III. show the same sort of figures for the iodine value of the fatty acids in the liver, higher, that is, than any that ever have been observed under other conditions, *and even higher than those for the oil given.* The subcutaneous fat tends to be affected in the same sense, but to nothing like the same extent nor so quickly. In the case of the liver the effect is already well marked within

TABLE II.—*Showing Effect on the Liver Fat in Rats of Feeding with Cod Liver Oil.*

Rat.	Weight.	Duration of feeding.	Approximate amount of oil eaten.	Liver.		Skin.	Rest of body.
				Fatty acids.	Iodine value of fatty acids.	Iodine value of fatty acids.	Iodine value of fatty acids.
No.	Grms.			%			
1	150	0	0	1·7	135	84	108
2	154	< 16 hours.	3 c.c. (much food in stomach).	2·3	131	100	112
3	200	*48* hours.	*20* c.c.	4·9	*215*	83	—
4	196	4 days.	7 c.c.	4·9	184	103	130
5	226	6 ,,	21 c.c.	2·8	177	94	132
6	161	7 ,,	14 c.c.	2·7	167	88	136

Iodine value of fatty acids from cod liver oil, 150.

TABLE III.—*Showing Effect on the Liver Fat, as seen within 24 Hours, of Feeding with Different Fats or Oils.*

Rat.	Weight.	Amount of jelly eaten.	Fat or oil eaten.	Liver.		Skin.	Rest of body.
				Fatty acids.	Iodine value of fatty acids.	Iodine value of fatty acids.	Iodine value of fatty acids.
No.	Grms.	Grms.		%			
1	125	50	0	2·8	139	87	122
2	168	8	< 1 gramme ol. morrh. (iodine value 150).	6·5	146	72	79
3	186	25	4·5 grammes ol. morrh.	4·8	173	75	86
4	237	30	5·5 grammes ol. morrh.	4·4	173	85	86
5	187	40	8 grammes margarine (iodine value 40).	4·8	133	71	76
6	203	25	5 grammes cocoa butter (iodine value 8).	5·1	113	74	80

The rats were kept without food for 24 hours, then each was given 50 grammes of jelly prepared with or without fat, and killed 24 hours later. All these rats were very well nourished and fat before the experiment began.

24 hours of the beginning of the feeding. The contrast between the rats that had cod-liver oil and those that had none is constant, and still more marked with those that had fat of a low iodine value, cocoa-butter. The other organs of a rat are too small to be satisfactorily worked in this way.

In order to get figures for other organs it is necessary to take a larger animal. Cats, of course, take fish greedily, and it is well known that herring oil, the fat of the muscles, forming in the fish I examined about 8 per cent. of the fresh tissue, has a high iodine value, that which I examined 127. But the most remarkable fatty acids appear to be obtained from the ovary of this fish. The samples analysed contained about 5 per cent. of fatty acids with the iodine value falling between 180 and 190.

No. I.—A cat was given three meals consisting respectively of 108 grammes and 210 grammes of herring roe and 90 grammes of herring flesh rubbed to a paste with 10 cubic centimetres of cod-liver oil. It was killed 40 hours after the first meal.

No. II.—Another cat ate at its first meal 95 grammes of herring roe, at its second 130 grammes of herring flesh with about 12 cubic centimetres of cod-liver oil, and at its third 143 grammes of herring roe. It was killed 60 hours after the first meal.

No. III.—A cat fed on 100 grammes of cod flesh daily with no fat for four days, then given one meal of herring roe. After four hours the cat was killed, the contents of its stomach removed, and the amount of fat in this determined. The amount of fat in the food eaten was known, the difference gives the maximum amount of fat that can have been absorbed—namely, 2·3 grammes. The contents of the intestine were not collected or analysed but certainly contained a part of this 2·3 grammes not yet absorbed.

No. IV.—A cat fed on 100 grammes cod flesh daily with no fat for five days, then given one meal of herring roe and killed eight hours later. The fat in the food eaten, less the fat in the food found still in the stomach, amounted to 3·3 grammes. Probably the amount actually absorbed was considerably less than this.

No. V.—A cat fed on 100 grammes cod flesh daily with no fat for five days, then given about 24 cubic centimetres cod-liver oil with its food in the course of 40 hours and killed 16 hours after the last meal.

Four normal cats, A to D, fed on horseflesh and milk were examined for comparison, and one, E, that had 100 grammes of cod flesh daily and no other food for five days.

The figures given in the table show that the liver is the only organ of those examined in which the result under consideration is noticeable. The oil finds its way, much the larger part of it no doubt to the connective tissue depots, and tends to raise the iodine value of the fat already there

TABLE IV.—*Showing Amount and Nature of Fats in the Organs of Normal Cats A to E, and Cats fed on Fish Oils I. to V.*

	Liver.		Heart.		Spleen.		Kidneys.		Adipose tissue.
	Fatty acids.	Iodine value of fatty acids.	Fatty acids.	Iodine value of fatty acids.	Fatty acids.	Iodine value of fatty acids.	Fatty acids.	Iodine value of fatty acids.	Iodine value of fatty acids.
	%		%		%		%		
A.	2·54	137·7	2·26	125·4	1·96	103·9	4·3	73·1 73·0	69·7
B.	2·22	129·4	2·99	127·8	—	—	6·0	61·1 61·2	—
C.	3·95	136·9	3·06	119·1	—	—	4·6	72·8	73·5
D.	22·25	94·5	3·01	102·2	—	—	6·8	66·7	61·25
E. Fed on cod flesh for 5 days.	19·2	90·6	2·55	118·5	1·93	116·5	5·4	77·4	59·1
I. Herring roe 40 hrs.	2·96	181·7	3·58	116·4	2·31	140·0	3·7	100·3	94·8
II. Herring roe 60 hrs.	5·74	170·7	3·00	120·7	2·27	121·6	—	—	88·2
III. Herring roe 4 hrs.	2·58	152·5	3·08	112·0	1·95	118·0	3·97	80·5	67·5
IV. Herring roe 8 hrs.	3·50	166·9	2·33	137·9	2·62	106·0	—	—	84·6
V. 24 c.c. ol. morrh. in 40 hrs.	4·04	185·0	2·21	130·3	—	—	—	—	73·8

with which it mixes. The same thing happens in the liver, both in cats and rats, and, as the experiments with cats show, not on the same scale at any rate in any other organ; not at all, for instance, in the heart, an organ in which everything tends to show that fat is used as a source of energy very largely. And in the liver the effect is much more marked than in the adipose tissue because the liver is not a place to which fat comes to stay, or in which it is stored. The character of the fat in the liver is determined not necessarily by the character of the fat which has been eaten and stored by the animals in weeks past, but by the character of the fat that has recently been circulating in the blood. This, of course, may in times of mobilisation be fat from the stores in the adipose tissue. But when there is abundant fat taken up from the intestine mobilisation of stored fat does not occur; the animal does not get thinner but fatter if plentifully fed. In this case the liver takes up the food fat and in the liver this food fat is more obvious than in the connective tissues, because in the liver it is not diluted down with fat eaten, it may be, weeks or months ago, since fat is not stored in the liver, but disappears from it to make place for other fat that it can get from the blood in its stead. The character of the fat in the liver, therefore, is determined in the first instance by the character of the fat offered it by the blood.

But in the liver there is something more than this. If this were all that happened the iodine value of the acids might approximate to that of the cod-liver oil acids but could not exceed it. In all the rats it exceeds it and also in Cat No. V., which is the only cat that is strictly comparable. This can only mean that the liver has the power of desaturating fatty acids and exerts it even when the acids supplied are already as unsaturated as those of cod-liver oil. Why even in this case the change is brought about we can at present only speculate. We know that the properties of unsaturated fatty acids of the same empirical formula vary according to the position of the unsaturated linkage. For instance, the ordinary oleic acid has the double bond in the middle between the ninth and tenth carbon atoms. Le Sueur prepared synthetically the acid with the double bond between the sixteenth and seventeenth, or, to use the ordinary nomenclature, between the α and β carbon atoms. This acid would not decolourise bromine water.[15] In certain positions, then, the double linkages are more stable than in others. It may therefore be that the liver is in the habit of removing hydrogen and introducing double bonds in certain positions in the chain and does so whether there are such unions in other positions or no. If the acids of the fish oils are unsaturated in the wrong places they might then become still more unsaturated when brought within the sphere of action of the liver. On the other hand, it may be that there are double unions in the acids of cod-liver oil which do not absorb iodine as readily as they would in other positions in the chain—are not, that is, so liable to oxidation. The action of the liver may be to move these double linkages along the chain into positions in which they not only absorb iodine more readily, but are more liable to the disruptive oxidising action of the cells that have to depend on fat for the supply of energy. The one idea involves the formation of new double bonds, the other a transposition of existing ones.

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[15] Journal of the Chemical Society, 1904, p. 1708.

However that may be, the conclusions which are indicated by the observations described are, in the first place, that the liver has after all something to do with the fat absorbed from the intestines. Its position gives it, it is true, no special privileges in this respect. The absorbed fat being conveyed into the systemic circulation, other parts of the body have an equal chance of taking it up as it is brought to them in the arterial blood. So far as we know at present of these other parts of the body it is only the connective tissues that avail themselves of the opportunity. And they take the fat up as it comes to them and store it unchanged. The liver, on the other hand, not only takes up its quota, but gets to work on it at once, and brings about changes in the acids, for which the term "desaturation" may be convenient, though it must be remembered that sometimes possibly the change may be merely a transposition of unsaturated linkages, while in other cases the process must apparently be of the kind which the term desaturation implies.

I think it will be admitted that support is thus gained for the idea that the liver has work to do in preparing fat for the metabolic processes in which fat is concerned. There are no *a priori* grounds for thinking that this work is more than of a preparatory nature; the complete oxidation of fat liberates so much energy that the later intensely exothermic stages of the breakdown of fat are likely to be reserved for organs where the discharge of energy is conspicuous—the heart, for instance, or the muscles or kidneys. If there is an extensive discharge of energy in the liver it is curious that it is so difficult to say what form that discharged energy takes.

It looks as if the work of the liver consisted in an operation which may be compared to the drying of gunpowder. The fats we take in our food are remarkably unreactive substances, and it has always been one of the most astonishing chemical achievements of animal cells that they should be able to burn up completely and cleanly as they do so stable a structure as saturated fatty acids like palmitic or stearic acids. They are wet gunpowder. And the body stores its gunpowder wet, and safely removed from the inflammatory operations of busily working cells. When the orders for mobilisation are issued this wet powder is conveyed to the drying chambers in the liver, and from there distributed to the fighting line in a proper condition for use. There are times when the stress of this work is manifestly too great. Too active a mobilisation of stored fat, or too little activity in dealing with it on the part of the liver, will result in an accumulation of the unfinished product in that organ. A fatty liver is then the result, and the fatty acids which it contains are found to have a low iodine value. There is an accumulation of wet powder.

Some of the conditions in which such accumulations are found appear to fit in with this conception of the way in which they come about. Pflüger, in his account of an important experiment on pancreatic glycosuria, noted that the dog which had excreted more than three kilogrammes of sugar in the course of two months, during which it was given no fat or carbohydrate, was at the end emaciated to such a degree that no fat could be found stored anywhere in its body. Nevertheless, its liver had not wasted and contained a normal amount of fat, 2·7 per cent. Hence he concluded that the liver keeps itself in a state of nutrition in diabetes, as the heart does in all forms of starvation, because of its activity which consists according to him largely in converting into sugar the fat brought to it from the storage places for fat. So far as the transportation of fat is concerned we must, I feel, be with Pflüger; as to the way in which fat is worked up in the liver, Pflüger's position is one that is at any rate questionable. The fatty livers that occur not uncommonly in diabetes and the condition described by Rosenfeld in dogs poisoned by phlorrhizine would be intelligible if interpreted as the result of excessive activity in the mobilisation of stored fat due to the necessity in which the body finds itself owing to the draining away of so much carbohydrate. The mobilisation of fat must, of course, occur always in starvation. Mottram has shown that when this occurs the amount of fat in the liver is increased. A preliminary note by him on this subject[16] appeared 18 months ago. His proof since then has become far more circumstantial and convincing, and though this valuable work is not yet published I have his permission to refer to it here. He experimented by feeding rabbits and guinea-pigs for a long period on a constant liberal diet so as to have a number of animals in as nearly the same condition of nutrition as possible. Some were killed to serve as normal controls and others after one, two, three, four, or five days' starvation. Evidence was obtained in this way that an importation of stored fat to the liver occurs after one day and again after four days' starvation. At these periods not only is the amount of fat in the liver increased, absolutely as well as histologically, but the iodine value of the fatty acids is lowered. In the intervals the importation ceases, the liver being at work on that which has been brought to it, the amount of fat in it diminishing and the iodine value rising.

It is not, of course, to be expected that active mobilisation of fat should necessarily and in all cases outrun the powers of the liver for working up and handing on the fat to other parts. A tendency for the liver to be in arrears of work is all the evidence we can expect from the examination of the organs of starving animals. Just as in the matter of alimentary glycosuria, we must expect to find individual differences.

On the grounds, therefore, which I have given I think it is a fair hypothesis to make the starting point for work that the liver stands in a different relation to circulating fat from the other organs of the body. Its work is to take up this fat and bring about certain changes in it, the result of which is to make this material available for the use of the organs in which its potential energy is required. I have described the grounds we have for thinking that this change consists, at any rate in part, of a desaturation of the fatty acids. Is that the whole of the change? There is another difference between what may be called organised fat—the fat, that is, in the cells of the heart, muscles, kidneys, &c.—and the unorganised merely stored fat of the adipose tissue. The latter is the unaltered fat of the individual's food ; it has its iodine value determined by that of the fat which has been absorbed, but it also contains no phosphorus. The fat in the liver and the other organs of the body, the organised fat, is very largely composed of phosphatide lipoid substances. Of these we know more or less dimly of a considerable number, more or less definitely of one lecithin. Rubow estimates roughly that about a half, or sometimes more than a half, of the organised fat is "lecithin."[17] It has also been repeatedly noted that the fatty acids which can be obtained by saponification of lecithin or other phosphatide lipoid substances are more unsaturated than those from adipose tissue.

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[16] Journal of Physiology, vol. xxxvi., p. viii.

It may therefore be that the work of the liver consists in the conversion of food fat into organised fat and that the desaturation of fatty acids is a change which occurs after that conversion has taken place ; a change that is, therefore, removed from our ken so long as our knowledge of the nature and constitution of these lipoid molecules is as meagre as it is at present. The change may be brought about behind a veil within the mysterious circle of influence of the phosphoric acid and the nitrogenous basic constituents of these more complex molecules. In order to ascertain whether this is so Kennaway examined the acids of a number of extracts which I had prepared in fractions from normal and pathological livers.[18] The tissue was extracted repeatedly with alcohol, and then repeatedly with ether, in the cold. The solvents were evaporated off completely at a low temperature and pressure and the residue taken up in dry ether and filtered. The ethereal solution was treated with acetone, which, broadly speaking, precipitates phosphatides but dissolves simple glycerides or ordinary fats. The precipitate contains nitrogen and phosphorus in about the amounts found for the best characterised lipoid complexes or mixtures of these ; the acetone solution contains these elements in about one-tenth of those amounts. The precipitated substances when saponified yield insoluble higher fatty acids, soluble in light petroleum, in amounts which fall within the limits of the amounts in known lipoids, less, at any rate, than 70 per cent. The unprecipitated substances contain amounts of these acids which approximate to those found in pure simple glycerides more or less closely—that is to say, to about 95 per cent. But the iodine value of the fatty acids

[17] Archiv für Experimentelle Pathologie und Pharmakologie, vol. lii.

[18] THE LANCET, Jan. 9th, 1909, p. 95.

obtained from the simple glycerides was invariably higher, and generally considerably higher, than that of the acids in adipose tissue fat. It was lower, it is true, than that of the acids in the lecithin fraction of the acetone precipitate, the fraction, that is, which is soluble in absolute alcohol, and perhaps still lower than that of the acids of the jecorine fraction, which is insoluble in alcohol as well as in acetone. It would seem, therefore, that even the simple glycerides in the liver are largely compounds of acids which have undergone the desaturation process; in so far as they are not, they consist of the raw material not yet dealt with in this way. The reaction involved, therefore, is not one that is carried out only in the lipoid molecules of which we know as yet so little but is comparatively accessible, and there is fair ground for hope that further work on the subject may bring to light much more that is definite with regard to it.

The same sort of change clearly has been carried out in the case of the fatty acids found in the liver of fish like the cod, which yield an oil with a high iodine value. A most interesting investigation into the nature of the acids contained in cod-liver oil has been carried out by Bull. He prepared the methyl esters of the fatty acids and distilled these in vacuo fractionally. From the different fractions he isolated, in addition to palmitic, stearic, and oleic acids, myristic, the saturated acid with 14 carbon atoms, and three other acids of the oleic series, one with 16 carbon atoms, clupeoleic, corresponding to palmitic in the saturated series, one with 20 called gadoleic and an erucic acid with 22 carbon atoms.

None of the acids isolated, however, account for the high iodine value of the mixed acids obtained by saponifying this oil, 150 or more. The isolation and characterisation of acids with more than one double link is a matter as yet of considerable difficulty. The most definite results have been obtained so far by oxidising the mixed acids with alkaline permanganate in the cold. Saturated acids are not attacked, unsaturated acids become saturated by the assumption of hydroxyl groups. Oleic acid thus becomes dihydroxystearic, linoleic becomes tetrahydroxystearic acid, and so forth. These hydroxy acids can be separated, purified, and identified, though the yields are not always very satisfactory. The further oxidation of these hydroxy acids on being heated with permanganate results in the cleavage of the acids into two parts, at the position of the original double bond. This is one of the ways in which ordinary oleic acid has been shown to have its double link exactly in the middle ; for the two acids produced each contained nine carbon atoms, one being a di- the other a mono-carboxylic acid ; thus

$$C_8H_{17}.CH = CH.C_7H_{14}.COOH =$$

Oleic acid.

$$C_8H_{17}.COOH + COOH.C_7H_{14}.COOH$$

Pelargonic acid + azelaic acid.

Another way of studying the unsaturated acids that has been much used is to saturate them with bromine and examine the products. The more bromine these contain, the more limited is the solubility in ordinary solvents.

Hartley has for some time been engaged on the study of the fatty acids present in various compounds in the liver of the pig. The results will be very shortly published in full, but some of the more striking results of his work may be alluded to here. On oxidising with permanganate in the cold a dihydroxystearic acid is obtained which has a different melting point from that of the dihydroxystearic acid obtained from ordinary oleic acid or from the pig's adipose tissue fat. On further oxidation this acid breaks up but does not yield pelargonic and azelaic acid as the oleic acid in, for instance, the omental fat of pigs does. Hartley obtained pelargonic acid from this latter, but with the same treatment of the dihydroxystearic acid from the pig's liver he obtained again and again no volatile fatty acid but caproic. This seems to imply that the double bond in the hepatic oleic acid is in a different situation, not in the middle but one-third of the way down the chain starting from the unoxidised end. This, then, would be an instance of the transposition of the double bond to which reference was made earlier in the lecture.

The constitution of none of the linoleic acids that occur in nature, the 18 carbon acids with two pairs of unsaturated carbon atoms, is known. It is not known, that is to say, where the double links occur ; there is obviously a large number of conceivable situations in which they might exist. Hartley has obtained from the fatty acids of the pig's liver one tetrahydroxystearic acid which melts sharply at a temperature a little above that given for sativic acid, the tetrahydroxystearic acid obtained from vegetable oils, and which on analysis gives figures agreeing exactly with the formula $C_{18}H_{36}O_6$. He has obtained also a certain amount of evidence indicating the presence of a second tetrahydroxystearic acid in the oxidation products of the liver fatty acids. This latter is more soluble in boiling water and has a lower melting point than the former. If possible it is hoped to identify the products of further oxidation of these acids derived from the linoleic acids in the pig's liver. It is these linoleic acids that seem to be the most abundant of the unsaturated acids in this organ and to be principally responsible for the high iodine value of the hepatic fatty acids.

But among the hydroxy acids which Hartley has obtained is a curiosity; one that from its solubility in water must evidently contain a large number of hydroxyl groups. It crystallises well, and melts sharply, and on analysis gives figures agreeing exactly with those for octohydroxy arachidic acid. This indicates the presence of an acid in the liver which has a chain of 20 carbon atoms and four double links. Further evidence of the occurrence of this acid he obtained in the formation of its octobromide on treating the acids with bromine. Anyone who has estimated the iodine value of the fatty acids from liver fats or oils must have noticed a precipitate that is formed by the iodine solution on reacting with the fatty acids. In the case of the acids from the pig's liver this precipitate appears to contain no hexabromide and to be composed entirely of this octobromide. This substance is insoluble in every solvent that has been tried, and is isolated only by extracting the bromination products successively with ether and benzene. This, of course, is not a very satisfactory way of arriving at a pure product. But several distinct preparations of the insoluble substance left have analysed correctly for octobrom-arachidic acid, and, taken together with the analysis of the oxidation product mentioned above, this seems to be fairly satisfactory evidence for the occurrence of this remarkable acid, $C_{20}H_{32}O_2$. Hartley estimates that it is present in fair amounts, and constitutes perhaps 8 to 10 per cent. of the acids in the pig's liver. An acid of the same formula is thought by Bull to be present in herring oil, whereas Tsujimoto obtained from this oil and from Japanese sardine oil an acid with 18 carbon atoms and four unsaturated links.

There are, indeed, clearly yet things to be learnt with regard to the fatty acids of the liver. But a beginning has been made and indications exist as to the nature of the biological significance of some of the facts that have already been determined. To assign its place in the metabolism of fats to this remarkable acid, however, is a task that we are not as yet equipped to undertake. It may be a product of that synthetic activity of which there is some evidence in the liver; but it is impossible to say. Small traces of arachidic acid occur in butter, and the corresponding alcohol, with 20 carbon atoms, has been found in the fat of dermoid cysts.

The more one works at the subjects on which I have had the privilege of addressing you this evening the more he becomes aware of the difficulties and obscurities that confront him. In view of these the few facts I have been able to muster present but a thin array which I have in vain tried to stiffen with thinner speculations. But a fact or two and a few changes of hypothesis constitute the journeyman investigator's stock in trade. And I have laid mine before you in the hope that you may be interested in them and in what I hope they may lead to.

Journal Physiology, Vol. XXXVIII.

[*From the Proceedings of the Physiological Society*, February 27, 1909.]

On the desaturation of fatty acids in the liver. By J. B. LEATHES and L. MEYER-WEDELL.

Some experiments on the effect which feeding different fats or oils would have on the nature of the fatty acids in the liver were commenced last summer by Dr L. Meyer-Wedell. The fatty acids in the liver of rats were found to absorb from 130 to 140 °/₀ of iodine. The fatty acids obtained from a sample of cod liver oil absorbed 150 °/₀. The livers of rats fed on this oil yielded fatty acids that absorbed 161, 198, 170, 173, 174 °/₀ of iodine. Dr Meyer-Wedell's experiments were interrupted by her having to return to Germany. They have been continued with the following results.

(1) Rats were fed with a homogeneous emulsion of cod liver oil in a jelly containing plasmon and ground rice, cooled down rapidly while still being shaken. In this way an approximate estimate of the amount of oil eaten could be made.

TABLE I.

Rat No.	Weight in gms.	Duration of feeding	Approximate amount of oil eaten in c.c.	Liver, Fatty acids per cent.	Liver, Iodine value of fatty acids	Skin, Iodine value of fatty acids
1	150	0	0	1·7	135	84
2	154	<16 hours*	3*	2·3	131	100
3	200	48 ,,	20	4·9	215	83
4	196	4 days	7	4·9	184	103
5	226	6 ,,	21	2·8	177	94
6	161	7 ,,	14	2·7	167	88

* Found dead in the morning, stomach containing much oil.

As in Dr Meyer-Wedell's experiments the fatty acids of the liver had a high iodine value higher even than that of the oil eaten: a remarkably high figure was given by No. 3 which had eaten a large amount of oil in a short time. No. 2 probably died before much if any oil had been absorbed.

(2) Six rats were given the same amount of the same jelly to which in one case no fat or oil was added, in three cases cod liver oil, in one case margarine and in the last cocoa butter. The iodine value of the fatty acids in the margarine was 40, in cocoa butter 8. The animals were all killed 24 hours after beginning to feed on this food.

TABLE II.

Rat No.	Weight	Amount of jelly eaten	Amount of fat or oil eaten	Liver, Fatty acids per cent.	Liver, Iodine value of fatty acids	Skin, Iodine value of fatty acids
7	125	50 gms.	0	2·8	139	87
8	168	8 ,,	<1 gm. Ol. M.	6·5	146	72
9	186	25 ,,	4·5 gms. Ol. M.	4·8	173	75
10	237	30 ,,	5·5 ,,	4·4	173	85
11	187	40 ,,	8 gms. Margarine I. value, 40	4·8	133	71
12	203	25 ,,	5 gms. Cocoa Butter I. value, 8	5·1	113	74

The same result as in the previous experiments is seen within a shorter period, 24 hours (Nos. 9 and 10): whereas when the food contains the usual sort of fat (No. 11) the usual iodine value is found in the liver fatty acids, and when a fat containing an abnormally small amount of unsaturated acid is fed (No. 12) the iodine value of the fatty acids in the liver is abnormally low.

The inference may be drawn that a portion of the fat absorbed from the intestine is taken up at once by the liver.

(3) That the other organs of the body do not, like the liver and connective tissue, take up the fat absorbed from the intestine is shown by the following experiments on cats.

The cats A, B, C and D were controls that had had no special dietary preparation: E was a control fed for five days on cod flesh which contains mere traces of fat.

The cat No. 1 had three meals consisting respectively of 108 grms. and 210 grms. of herring roe and 90 grms. of herring flesh rubbed to a paste with 10 c.c. of cod liver oil, and was killed 40 hours after the first of these meals was given.

The ovary of the herring contains about 5 % of fatty acids the iodine value of which falls between 180 and 190. The flesh of the herring contains about 8 % of fatty acids with the iodine value 127.

The cat No. II ate at its first meal 95 grms. of herring roe: at its second 130 grms. of herring flesh with about 12 c.c. of cod liver oil and at its third 143 grms. of roe. It was killed 60 hours after the first meal.

Nos. III, IV and V had no food but 100 grms. of cod flesh daily for 5 days and then were given respectively:

No. III a meal of herring roe, from which it absorbed not more than 2·3 grms. of fatty acids, all the rest being found in the stomach when it was killed 4 hours after the meal. The contents of the intestine were not collected or analysed.

No. IV also had a meal of herring roe and when killed 8 hours later had absorbed not more than 3·3 grms. of the fatty acids.

No. V took 24 c.c. of cod liver oil mixed with herring flesh and was killed 40 hours after the first meal of this food and 16 hours after the last.

TABLE III.

Fatty acids in the:

	Liver		Heart		Spleen		Kidneys		Skin
Cats	per cent.	Iodine value	per cent.	Iodine value	per cent.	Iodine value	per cent.	Iodine value	Iodine value
A	2·54	138	2·26	125	1·96	104	4·3	73	70
B	2·22	129	2·99	128	—	—	6·0	61	—
C	3·95	137	3·06	119	—	—	4·6	73	73
D	22·25	94	3·01	102	—	—	6·8	67	61
E	19·2	91	2·55	118	1·93	116	5·4	77	59
I	2·96	**182**	3·58	116	2·31	140	3·7	100	95
II	5·74	**171**	3·00	121	2·27	122	—	—	88
III	2·58	**152**	3·08	112	1·95	118	4·0	80	67
IV	3·50	**167**	2·33	138	2·62	106	—	—	85
V	4·04	**185**	2·21	130	—	—	—	—	74

These figures show: that feeding with fish oils of a high iodine value results in the appearance of fatty acids with a similarly high iodine value in the liver but not in the other organs examined: this result in the liver is most marked when the feeding is repeated a second or third time (Nos. I, II and V) but is also evident within a few hours of a single meal and after the absorption of small amounts of fat (Nos. III and IV). As in rats so too in the cat feeding with cod liver oil results in the appearance in the liver of fatty acids more unsaturated even than those of the oil fed.

The experiments show (1) that the liver as well as the connective tissues takes up fat conveyed to it by the blood. The other organs do not at any rate to the same degree. (2) But whereas the connective tissues store the fat as brought to them, the liver changes the fatty acids in such a way as to increase their power of absorbing iodine. This may be interpreted as due either to the introduction of new unsaturated linkages, or possibly to the transposition of existing ones from situations in which they are less liable to saturation by halogens (or hydroxyl groups) to others in which they are more so. It is known for instance that $\Delta^{\alpha\beta}$ oleic acid does not decolourize bromine water[1].

These facts furnish additional evidence for the view[2] that it is a function of the liver to prepare fat for oxidation in other tissues and that the preparatory treatment consists in part at any rate in a desaturation process.

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[1] Le Sueur. *Journ. Chem. Soc.* LXXXV. 1708, 1904.

[2] *Problems in Animal Metabolism*, pp. 107 and 118.

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The Influence of Temperature on Phagocytosis.

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(Communicated by Dr. C. J. Martin, F.R.S. Received November 18, 1907,—
Read February 27, 1908.)

Recent work has shown that the phagocytosis of micro-organisms is a complex phenomenon, involving at least two factors, viz.: (1) the sensitisation of the micro-organism by the opsonin of the blood serum, and (2) the phagocytic act of the polymorphonuclear leucocyte. The latter function has been known for many years to be markedly influenced by temperature. It appeared desirable, however, to ascertain whether, by the modern quantitative methods employed in opsonic work, some definite relationship could be found to subsist between degree of temperature and degree of phagocytosis. In the first set of experiments no attempt was made to separate the respective functions of sensitisation, and amœboid activity of the leucocyte. Both actions were allowed to proceed simultaneously.

Consequently, in Series I the leucocytes were put in contact with fresh serum and micro-organisms (staphylococci), and incubated together at different temperatures for the same period of time. Thereafter the number of cocci taken up per leucocyte was calculated on stained films in the usual way. The *Staphylococcus aureus* employed was an old laboratory strain.

Series I.—Experiment I.

Temperature.	Cocci per leucocyte with incubation periods.		
	15 mins.	30 mins.	45 mins.
43° C.	15·4	30	30+
37	13·2	23	25
18	3·1	3·3	7·9

It will be seen that at a temperature of 18° C. the degree of phagocytosis for 30 minutes is not appreciably greater than that for 15 minutes. There is, in fact, a prolonged latent period. This was further brought out in the next experiment, where the temperature remained constant (18° C.) while the incubation periods varied.

Experiment II.

Temperature.	Incubation period.	Cocci per leucocyte.
18° C.	15 mins.	2·9
18	30	3·8
18	45	7·2
18	1 hr.	10·7
18	1 hr. 15 mins.	12·7
18	1 hr. 30 mins.	19·2

For 37° C. this latent period is much shorter. To ascertain this, five-minute intervals were taken as in the following experiment:—

Experiment III.

Temperature.	Incubation period.	Cocci per leucocyte.
37° C.	5 mins.	2·0
37	10	2·7
37	15	4·3
37	20	4·0
37	25	5·6
37	30	4·9
37	35	5·0
37	40	6·5
37	45	8·6
37	50	12·0

The five following experiments showing the influence of temperature on phagocytosis were carried out in a similar way to Experiment I. Coccal emulsions of varying strengths were purposely employed throughout the series:—

Experiment IV.

*I. T. = 15 mins.

Temperature.	Cocci.
41° C.	17·1
37	15·3
17	4·2

Experiment V.

I. T. = 15 mins.

Temperature.	Cocci.
43° C.	15·7
37	12·3
31·5	10·9
25·7	4·0
20	3·2
13	0·8
6·5	?

Experiment VI.

I. T. = 30 mins.

Temperature.	Cocci.
42° C.	8·1
37	9·0
30	8·5
25	6·3
17	3·7

Experiment VII.

I. T. = 33 mins.

Temperature.	Cocci.
43° C.	28·0
37	20·7
31·5	22·3
25·7	18·4
20	10·9
13	9·6
6·5	0·7
0	?

Experiment VIII.

I. T. = 30 mins.

Temperature.	Cocci.
41° C.	21·4
36·6	11·8
30	8·2
17	4·8

From the results quoted above, it will be apparent that the degree of phagocytosis rises, though in a somewhat irregular fashion, as the temperature rises, but the results are not sufficiently constant to enable one to determine with any accuracy such a value for the temperature-coefficient as would allow one to calculate the amount of phagocytosis at a temperature A when the amount at a temperature B is known. The strength of the

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* I. T. = Incubation Time.

coccal emulsion has probably a great bearing on the rate of fall, especially during the upper temperature ranges (*vide* Experiment VI). A fairly constant ratio, however (4—5 : 1), is found to exist between the degree of phagocytosis at 43° C. and that at 18° C.

We have now to determine whether this increased phagocytosis following rise of temperature is due simply to increased activity on the part of the leucocyte. In short, what effect has temperature on the rate of combination of the serum with the coccus?

To settle this point, a second series of experiments was performed. The cocci were sensitised by contact with serum for a fixed time at a certain temperature. Thereafter leucocytes were added and incubation performed at different temperatures. By this method more or less completely sensitised cocci were exposed to the action of the leucocytes.

Series II.

Experiment I.—A moderately thick emulsion of cocci in normal saline was added in equal volume to fresh serum, and kept in contact therewith at a temperature of 37° C. for 20 minutes in a small test-tube. One volume of leucocytes was then incorporated with one volume of the shaken up and partially centrifugalised serum-coccal mixture and incubated at different temperatures for 20 minutes. The result was as follows:—

Temperature.	Cocci per leucocyte.
37° C.	4·4
30	4·4
17	4·3

(The low counts obtained here show that the centrifugalisation of the serum-coccal mixture had been carried a little too far.)

Experiment II.—Technique similar to above.

I. T. = 30 mins.

Temperature.	Cocci per leucocyte.
41° C.	13·8
37	11·8
30	12·2
17	13·5

(Again the fall of temperature had no effect on the amount of phagocytosis.)

Experiment III.—Combination at 37° C. for 20 mins.

I. T. = 15 mins.

Temperature.	Cocci.
37° C.	12·5
17	10·3

Experiment IV.—Combination at 37° C. for 20 mins.

I. T. = 15 mins.

Temperature.	Cocci per leucocyte
37° C.	5·7
28	5·2
18	5·9
7	3·6

Experiment V.—In this experiment the sensitisation was performed at 18° C. for 20 minutes, and the same coccal emulsion was employed as in Experiment IV, so that the figures obtained are quite comparable.

Temperature.	Cocci per leucocyte.
37° C.	6·2
28	4·2
18	2·6
7	0·8

Experiment VI.—A portion of the coccal emulsion was combined with serum at 37° C. for 20 minutes, and an equal portion combined with serum at 0° C. for the same time. Leucocytes were then added, and the results of phagocytosis at different temperatures compared.

Combination at 37° C.			Combination at 0° C.		
Temperature.	Incubation time.	Cocci.	Temperature.	Incubation time.	Cocci.
37° C.	15 mins.	12·1	37° C.	15 mins.	8·8
20	15	8·6	20	15	0·4

The very great difference between the amounts of phagocytosis at 37° C and 20° C. after combination at 0° C. offers a marked contrast to what obtains under the same conditions after combination at 37° C. The employment of very thin coccal emulsions, however, and the prolongation of the period of combination with the serum, have the effect of equalising the amounts of phagocytosis at high and low temperatures even after low-temperature combination.

Throughout the experiments which illustrate this point, thin coccal emulsions were combined with serum for long periods, and the mixtures required no centrifugalising before use.

Experiment VII.—Combination at 37° C. for 45 mins.

I. T. = 15 mins.

Temperature.	Cocci per leucocyte.
37° C.	9·6
18	8·0

Experiment VIII.—Combination at 37° C. for 2 hrs. 45 mins.

I. T. = 15 mins.

Temperature.	Cocci per leucocyte.
37° C.	15·0
18	14·5

Two further experiments similar to Experiment VIII gave ratios of 12·2 : 12·6 and 14·1 : 13·3 for the amount of phagocytosis at 37° C. to that at 18° C.

Experiment IX.—Comparison of the Effects of Combination at 37° C. and at 18° C.

Time of combination in each case, 1 hr. 40 mins. I. T. = 15 mins.

Combination at 37° C.		Combination at 18° C.	
Temperature.	Cocci.	Temperature.	Cocci.
37° C.	8·6	37° C.	4·9
18	8·5	18	6·1

It will be seen that after combination at 18° C. the amount of phagocytosis at 37° C. is even slightly exceeded by that at 18° C., though both fall very short of the corresponding figures obtained after combination at 37° C.

Experiment X.

Technique similar to that of Experiment IX. Temperatures of combination, 37° and 7° C. Time of combination, 3 hrs. 15 mins. Time of incubation, 20 mins.

Combination at 37° C.		Combination at 7° C.	
Temperature.	Cocci.	Temperature.	Cocci.
37° C.	23·0	37° C.	6·6
21	28·6	21	5·8
18	27·8	18	6·8
10	12·0	10	4·0

In each series, between the temperature range 37° C. to 18° C., the phagocytic values are practically identical, but the great differences between the absolute amounts in the two series afford a striking illustration of the superiority of combination at 37° C.

Experiment XI.—This experiment was designed to show the effect of varying the combination times at the two temperatures 37° C. and 18° C.

Samples for phagocytic purposes were removed from the serum-coccal mixtures every half hour and exposed to the action of the leucocytes.

Combination at 37° C.			Combination at 18° C.		
Temperature.	Combination time.	Cocci.	Temperature.	Combination time.	Cocci.
37° C.	30 mins.	5·0	37° C.	30 mins.	4·7
18	30 mins.	4·1	18	30 mins.	5·4
37	1 hr.	8·4	37	1 hr.	5·7
18	1 hr.	9·2	18	1 hr.	7·3
37	1 hr. 30 mins.	8·9	37	1 hr. 30 mins.	7·4
18	1 hr. 30 mins.	8·4	18	1 hr. 30 mins.	7·0
37	2 hrs.	10·7	37	2 hrs.	8·3
18	2 hrs.	10·5	18	2 hrs.	7·2

The pairs of phagocytic values in the various time intervals approximate very closely in the two series, showing that, under the conditions indicated, the degree of phagocytosis is independent of the temperature within the range 37° C.—18° C. Further, when we compare the figures in the two series, those of the right hand series (combination at 18° C.) decidedly lag behind those of the left hand series (combination at 37° C.). The difference, however, is not nearly so marked as in a previous experiment, where the effects of combination at 37° C. and 7° C. were compared. One point is somewhat difficult to explain. In the case of combination at 18° C. it was found in an earlier experiment where thick emulsions were employed that the amount of phagocytosis at 18° C. fell considerably below that at 37° C. In the present experiment, where thin emulsions were employed for combining purposes, these values are equalised. The reason probably is that, although the rate of combination at 18° C. is slower than that at 37° C., prolonged contact of serum with cocci at the lower temperature (18° C.) effects a maximum absorption of opsonin by the cocci for that temperature, and, consequently, when phagocytosis takes place, the influence of temperature within the range 37° C.—18° C. is reduced to a minimum, just as in the case of combination at 37° C. In a paper by Bulloch and Atkin (1905) it is stated that the combination of the opsonin with the micro-organism takes place as readily at 0° C. as at 37° C. If this were so, it would be difficult to explain many of the experimental results detailed above. Dean (1905), on the contrary, observed that combination at 0° C. was much slower than at 37° C. The problem was again attacked in the following way :—

Experiment XII.—Emulsions of dead tubercle bacilli were incorporated with equal volumes of serum at the temperatures of 37° C. and 0° C. for a period of 30 minutes. The mixtures were then completely centrifugalised and the opsonic contents of the supernatant fluids towards fresh tubercle bacilli compared, fresh normal serum, in corresponding dilution, being employed as control.

	Bacilli per leucocyte.
Supernatant fluid after combination at 37° C.	3·4
„ „ „ 0° C.	6·4
Fresh serum (diluted)	6·2

Experiment XIII.

Here the period of combination was prolonged for 11 hours.

	Bacilli per leucocyte.
Supernatant fluid after combination at 37° C.	2·5
„ „ „ 0° C.	6·7
Fresh serum (diluted)	5·0

Experiment XIV.

Period of combination, 1 hr. 45 mins.

	Bacilli per leucocyte.
Supernatant fluid after combination at 37° C.	1·4
„ „ „ 0° C.	5·1
Fresh serum	7·1

These were the figures obtained after phagocytosis for 15 minutes. When phagocytosis was prolonged to 30 minutes the corresponding values obtained were 1·6, 11·3 and 10·9. The supernatant fluid, after combination at 37° C., had therefore been exhausted of its available opsonin after 15 minutes, whereas in the case of the second supernatant fluid (combination at 0° C.), the prolongation of the incubation period from 15 minutes to 30 minutes had the effect of more than doubling the phagocytic intake. Further, these fluids were tested against a fresh emulsion of staphylococci, when the following figures were obtained:—

0·9, 3·2, and 4·0 (control serum).

After combination at 37° C., therefore, the serum had lost nearly all its contents of tubercular and staphylococcal opsonins, whereas, after absorption at 0° C. little loss had occurred. It may be noted that incidentally the result tells against the specificity of the opsonin of normal serum.

There can be no doubt that absorption at 37° C. removes from a serum far more opsonin in the same time than absorption at 0° C. does. An attempt was made to test the degree of sensitisation of the centrifugalised bacilli after combination at 37° C. and 0° C. respectively. The supernatant fluids were removed and replaced by saline (twice repeated). When leucocytes were added to saline emulsions of the deposited bacilli, made up to the same volume and incubated at 37° C., little difference was found in the numbers taken up in the two cases. The technique is difficult and further experiments may have to be performed in this connexion, but if the phenomenon is constant it would show that at 37° C. the bacilli absorb more opsonin than is necessary for efficient sensitisation, while at 0° C. the bacilli remove just sufficient opsonin to prepare them for phagocytosis.

Summary and Conclusions.

1. When serum, cocci, and leucocytes are mixed directly and incubated at different temperatures, the number of cocci taken up increases more or less regularly with the temperature. By this method it has been shown that the phagocytic intake at 18° C. is only about one-fourth to one-fifth ot that at 37° C.

2. This fall, at least within the temperature range 37° C.—18° C., is due to the diminished rate of combination of the serum with the coccus as the temperature falls.

3. When cocci which have previously been exposed to the action of serum, either at 37° C. or at 18° C., are put in contact with leucocytes, the intake is practically the same whether the phagocytosis takes place at 37° C. or at 18° C. The number taken up, however, after combination at 18° C. and more especially at 7° C., falls very short of the number taken up after combination at 37° C.

4. Experimental results, detailed above, lead one to assume that prolonged contact of a serum with cocci at a low temperature (18° C. or 7° C.) leads to a maximum absorption of opsonin by the cocci (corresponding to that temperature), so that the subsequent phagocytosis is identical whether it takes place at 37° C. or at 18° C.

5. Provided that cocci loaded with opsonin up to a certain maximum are presented to the leucocyte, the phagocytic energy of the latter is independent of the temperature within a wide range.

6. From the appearances on stained films it would seem that sensitised micro-organisms exposed to the action of leucocytes at very low temperatures tend to congregate near the periphery of the leucocytes, although little or no phagocytosis may take place. Hence, within a suitable temperature range, it may be presumed that the inclusion of sensitised micro-organisms by the leucocyte is a surface tension effect taking place between the coccus and the protoplasmic wall, amœboid energy playing only a subordinate part in the process.

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THE TYPHOID CARRIER PROBLEM, WITH SOME EXPERIMENTS ON IMMUNITY IN CARRIERS.

Being a Paper read in the Section of Pathology at the Annual Meeting of the British Medical Association, Sheffield, 1908.

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During the last three or four years our knowledge of the pathology and epidemiology of typhoid fever has been considerably widened as a consequence of the discovery of the chronic carrier.

To Koch, who in 1903 instigated the bacteriological investigation of typhoid epidemics at their seat of outbreak and drew attention to the necessity of preventing contact infection (in other words, of searching for and finally isolating the temporary human host of the bacillus), is due the credit of initiating those researches which led up to the discovery of the chronic carrier.

Frosch, Dönitz, and others, working at their typhoid examination stations in south-west Germany, abundantly proved the danger due to contact infection. They remained puzzled, however, by several peculiar phenomena, notably that of the so-called "typhoid house." After the occurrence of a case of typhoid fever in a house which had hitherto been free from infection, it was frequently observed that cases would continue to crop up in the same house for long periods. In explanation of this Frosch suggested the possibility of a prolonged or indefinite excretion of the typhoid bacillus by the convalescent, but it was left for Conradi, Lentz, Kossel, and especially Forster, Kayser, and their collaborators of the Strassburg school, to demonstrate conclusively by painstaking bacteriological investigation the existence of the chronic carrier and the danger to the community resulting from him.

I do not propose here to detail the many epidemiological and bacteriological researches which have resulted in the discovery of carrier cases as the causal agents in epidemics either in Germany or in this country. My object is to collate briefly the main pathological and bacteriological facts which have been so far established in regard to carriers and the lessons they are bound to teach to

[548/08]

hygienists, public health authorities, and more especially the public at large. In the first place, I wish to refer to the presence of the typhoid bacillus in the excreta of actual typhoid cases, typhoid convalescents, and typhoid carriers. It has to be noted at the outset that the employment of special media for the isolation and the identification of intestinal organisms, notably those of Conradi-Drigalski, Loeffler, and Endo on the Continent, and that of MacConkey in this country, have rendered the search for the typhoid bacillus in the excreta a comparatively easy task. Still, in spite of this great improvement in technique, it is important to remember that only in 20 to 30 per cent. of acute typhoid cases during the febrile period has the typhoid bacillus been isolated from the faeces. Klinger, in a series of 428 cases, got a percentage of 31.8. Others have got percentages as low as 15. On the other hand, the bacillus has been isolated from the blood in 90 per cent. of cases during the first week of the disease. From the urine the percentage of successes is about 20 to 25.

In the case of typhoid convalescents examined during the first few weeks after the cessation of the fever it has been found that about 10 to 13 per cent. are still excreting the bacillus, but of this 10 to 13 per cent. only 1.5 to 2 per cent. continue to excrete for a longer period than six weeks, and consequently fall into the category of chronic carriers. The figure obtained by Brion and Kayser from a series of 200 cases was 1.5, while Lentz got the considerably higher percentage of 4. The latter has been found to be much nearer the truth when the bacteriological investigation is deferred to a later period after convalescence. Thus, Kayser was able to collect a large number of cured typhoid cases at a period of two years after convalescence when they had been declared bacteriologically typhoid-free. It was found that, of the 101 cases so collected, 3 were still harbouring the bacillus, and were therefore chronic carriers.

Examinations on a large scale have also been made by the Strassburg School on healthy persons living in the vicinity of or in close contact to typhoid cases, and the surprising fact has been elicited that there is another type of carrier—the so-called temporary or acute carrier, who excretes the typhoid bacillus in small numbers for a short time only. Of course, in view of the intermittency observed in the excretion of the typhoid bacillus by chronic carrier cases, it might be thought that the disappearance of the bacilli in the temporary carriers coincided with an intermittent period. Further, it might be argued that these acute cases were really suffering from a mild ambulant form of the disease, but in any case the fact remains that all subsequent bacteriological investigations in these temporary carriers proved negative. As a striking illustration of the so-called temporary carrier the experience of Scheller in a recent milk epidemic may be quoted:

A female, engaged in the milk trade, was found to be excreting the typhoid bacillus, and there appeared to be no doubt that she constituted the focus of infection in the epidemic in question. All persons in any way associated with the milk trade, either as milkers, consumers, etc., were examined, with the result that 17 other carriers were revealed. Some of these were passing the bacillus in the faeces only, others in the urine only, while in a certain proportion both the faeces and the urine were infective. This surprisingly large number of carriers suggested the idea that they might merely be temporary harbourers of the bacillus. Accordingly the female originally discovered was removed from her work in the dairy. Instructions were given to the milkers to wash their hands in lysol, etc., before milking. Further, those who were passing the bacillus in the urine were treated with urotropin. A month after these precautions had been put in practice all the carriers were again examined bacteriologically, with the result that all proved negative except the original female, who continued to pass the bacilli in large numbers.

There can be no doubt that the typhoid bacillus can be ingested without harm by certain individuals and passed out by the excreta. We are not yet in a position to explain why the ingestion may not be followed by symptoms. Individual susceptibility, virulence of strain, and magnitude of dose are all factors to be reckoned with in this connexion. It may be that we are at a parting of the ways in our conception of typhoid. The recent finding of the typhoid bacillus in the blood of cases which proved on *post-mortem* examination to be cases of tuberculosis (Busse and others) is perhaps apt to shake one's notion of the typhoid bacillus as the causative agent of enteric fever. Such findings demand the fullest investigation both from the bacteriological and epidemiological standpoint.

It may, then, be taken as established that about 3 to 4 per cent. of typhoid convalescents become chronic carriers, and that in the neighbourhood of typhoid cases a small percentage of the community may be temporary harbourers of the bacillus.

Some of these carriers may have had typhoid fever within the past year or six months, while others have been found excreting the typhoid bacillus at a period of thirty years and more after the attack. Indeed, it seems at present as if the proposition, "Once a carrier always a carrier," could be safely enunciated. How far this assertion may be modified by attempts at therapy of a medical or surgical nature will be discussed below. These carriers excrete the bacillus, as is well known, in an intermittent fashion. In certain cases which the writer has had under observation several months of intermission have been noted. It may be that the typhoid bacilli in a particular sample of faeces are so few that our routine methods fail to reveal them, and more especially when, as frequently happens, the typhoid colonies are found congregated together in a particular portion of the plate, suggesting that they had been lying originally on a single plug of mucus. But whether this intermittency is real or apparent, there is no question that during a period of active excretion of the bacillus enormous numbers are found on the plates, to the almost complete exclusion of lactose fermenters. Intermittency is certainly the rule, though Kayser was able to record a case which showed no intermittency over a very long period.

The Gall Bladder Question.

Female carriers predominate in the proportion of 3 to 1, and this brings us to a consideration of the gall bladder problem. As is well known, this ratio also represents the relative incidence of gall-stone disease in the two sexes. Many of these female carriers suffer from gall stones. Others, again, have been found on *post-mortem* examination to have gall stones which gave rise to no symptoms during life. As a matter of fact, only 10 per cent. of cholelithiasis cases present symptoms of gall stone, and it is interesting to note that only 10 per cent. of carrier cases show symptoms. The work of Blumenthal, Naunyn, Ehret and Stolz, Forster and Kayser, has shown that the typhoid bacillus may be associated with gall stones in persons who have presented no history of typhoid whatever. At surgical operations on the gall bladder the typhoid bacillus has frequently been found in the bile and even in the interior of the gall stones. It would seem that probably a large proportion of gall-stone cases which come to operation are really typhoidal in nature, and it is highly important that in all such cases bacteriological examination of the bile should be carried out. The association of a positive Widal reaction with cases of icterus has long been known, and the explanation is in all probability to be found in the typhoidal origin of these cases.

The evidence in favour of the view that the gall bladder is the seat of vegetation of the typhoid bacillus in carriers is thus very strong. Experimentally in animals it has been found possible to retain the gall bladder in an infected condition for long periods after the bacilli have disappeared from the blood and urine. Some have objected that liver complications in typhoid fever are very infrequent; but it seems certain that an infective cholangitis may be present without giving rise to icteric symptoms. Such a cholangitis is associated with an unhealthy state of the bile passages and gall bladder, and permits the growth of the typhoid bacillus for an indefinite period, though there may be little or no systemic disturbance. In the case of the carrier of twenty-nine years' standing recently reported by Dean, the temporary feeling of pain over the gall bladder region occurring once a year was the only clue which led to his being examined and declared a carrier. Still it must be admitted that the presence of the bacillus in the gall bladder will not altogether explain the intermittency, at any rate, for long periods. It can quite readily be conceived that the bacilli are only intermittently ejected from the gall bladder into the intestine in consequence of some local temporary obstruction from gall stones or otherwise, but it is difficult to explain why the bacilli should appear in almost pure culture in the faeces in so many instances and later completely disappear. Numerous problems relating to the defensive mechanism possessed by the intestinal epithelium

The Infectivity of Carriers.

Regarding the question of infectivity of carriers it is difficult at present to make any definite statements. The intermittency in the excretion of the bacillus will explain much. Some carriers, especially in recurring milk epidemics, have been infective for very long periods, some apparently have only quite suddenly become infective, while others, so far as can be ascertained, have not given rise to typhoid in other people at any time in their history. It seems likely that in the case of healthy carriers exercising proper personal hygiene the danger of infecting other persons may be reduced to a minimum, but it is none the less necessary to regard every carrier moving about among his fellows as a potential source of infection. So far as the writer's experience goes the typhoid strains isolated from carriers possess quite a fair degree of virulence for the guinea-pig, but it is quite likely that there may be a seasonal variation of virulence.

Mixed Infections.

The association of paratyphoid or dysentery bacilli with the typhoid bacillus in carrier cases opens up an extremely interesting and important field of investigation, but cannot be discussed at length in this paper. Such cases have frequently been reported in institutions where typhoid fever has been endemic, and it seems probable that their occurrence is due to promiscuous housing of paratyphoid, dysentery and typhoid cases.

Treatment.

Hitherto all attempts to render carriers typhoid-free by medicinal treatment have been unsuccessful. Salol and other so-called intestinal antiseptics—calomel, ox-bile, chloroform, milk diet, etc.—have all been tried, but the bacilli have not been found to disappear completely. The great difficulty lies in effectively controlling one's results. In view of the natural intermittency of excretion, the faeces must be examined for very long periods after the administration of the drug. Surgical interference directed to the gall bladder has apparently been followed by success in a few cases. The operations of cholecystostomy with drainage of the gall bladder, and even cholecystectomy, have been performed in a few carrier cases, although symptoms pointing to gall-bladder disease had been absent. A condition of cholelithiasis was invariably found. Some months after the operations it became impossible to demonstrate the typhoid bacillus in the excreta, and the Widal reaction disappeared. Hitherto reports of treatment by typhoid vaccines have not been published.

IMMUNITY IN TYPHOID CARRIERS.

I propose to conclude with a brief account of certain experiments made with the serums of carrier cases whose histories were detailed in the BRITISH MEDICAL JOURNAL, January 4th, 1908.

Agglutinins.—It is now well established that the serums of carrier cases give as a rule a positive Widal reaction (1 in 50 to 1 in 100), a fact which is of great importance from the diagnostic point of view. It must not be forgotten, however, that several cases have presented what would be returned as a negative reaction, although typhoid bacilli were readily demonstrable in the excreta. On the other hand, it has been shown that the Widal reaction may disappear for a time, to return again in a marked fashion. The periods during which the Widal reaction has failed have not, however, been found to synchronize with the periods during which the *Bacillus typhosus* is absent from the excreta.

It is not unlikely that the serums of these cases may give agglutination reactions which vary with the strain employed in the test. Thus on November 18th, 1907, the serums of two carriers "A" and "B," and a control case "C," were tested against three typhoid strains, namely, strain "A" (from carrier "A"), strain "B" (from carrier "B"), and strain "G" (laboratory strain in constant use for Widal tests).

Carrier "A" was found to give a marked reaction only with the laboratory strain. With the homologous strain and with the other carrier strain there was only a trace of agglutination after nine hours. On the other hand, Carrier "B" gave a marked reaction with all three strains. The control case gave no agglutination with any of the strains.

That the serum of "A" possessed suitable receptors for the strain "A" was shown by experiments on the opsonic power (see below), and, without postulating any relationship between agglutinin and the opsonic substance, one might presume that only the binding group of the agglutinin receptor remained, while the agglutinating or precipitating group had been lost for the homologous strain. Further experiments on this question are necessary.

Bactericidal Experiments.—The few recorded observations of the bactericidal power of the serum in carrier cases go to show that little or no variation from the normal standard occurs. The serums of the three carriers above mentioned have been tested on two occasions against homologous and other strains. The serums were inactivated and complemented with fresh guinea-pig serum.

Some increase in the bactericidal power of the carrier serums was found over that of the control serum, but the differences seemed to depend largely on variations in the resistance of the strains employed.

Opsonic Experiments.—Owing to distance, the serums were not available for experiment until the third day after

	"A" Strain.	"B" Strain.	Laboratory Strain.
	Bacilli per Cell.	Bacilli per Cell.	Bacilli per Cell.
Control serum ...	6.2	5.3	3.1
"A" carrier ...	27 appr.	50 appr.	4.7
"B" carrier ...	40 appr.	40 appr.	4.5

With the avirulent laboratory strain extra and intracellular lysis was so extensive that the counts of "A," "B," and control approximated closely.

With the carrier strains the serums of "A" and "B" show a great increase of opsonic substance.

The same serums were tested against a strain of the *Staphylococcus pyogenes aureus*, with the following result:

"A" serum	13.4 cocci per cell
"B" serum	10.1 ,, ,,
Control serum	12.1 ,, ,,

Thus, no essential difference was demonstrated between the serums with respect to the *Staphylococcus pyogenes aureus*.

Experiment II.

To show the effect of inactivating the serums and complementing with fresh normal human serum.

	Bacilli per cell.
1 vol. (fresh serum of "B"+saline)+"B" strain	16.4
1 vol. (inactivated serum of "B"+saline)+"B" strain	0.9
1 vol. (inactivated serum of "B"+normal serum)+"B" strain	19.9
1 vol. (normal serum+saline)+"B" strain	3.7

It will be seen that by inactivation the opsonic property of the serum "B" is almost completely destroyed. It is restored, however, by complementing with fresh normal serum, which of itself possesses little opsonic action towards the "B" strain.

The effect of inactivating and complementing is also seen in the following similar experiment:

Experiment III.

	Bacilli per cell.
1 vol. (fresh "A" serum+saline)+"A" strain	7.5
1 vol. (inactivated "A" serum+complement)+"A" strain ...	6.2
1 vol. (inactivated "B" serum+complement)+"A" strain ...	5.4
1 vol. (inactivated "C" serum+complement)+"A" strain ...	8.2
1 vol. (complement heated+fresh complement)+"A" strain ...	2.2

The results of the above experiments, then, and others not here recorded, go to show that the serums of carriers possess a high opsonic action towards the homologous strains. This opsonic property is almost completely destroyed by inactivation, but is restored by complementing with fresh normal serum. Recently Schöne has demonstrated by complement-deviation experiments of Bordet-Gengou type, the presence of an amboceptor in the serums of carriers, and it seems highly probable in the light of the above experiments that his amboceptor is an opsonic one. I append a bibliography, which may be useful to those interested in the subject of typhoid carriers.

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BILE SALT MEDIA AND THEIR ADVANTAGES IN SOME BACTERIOLOGICAL EXAMINATIONS.

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The addition of bile salts to media was first employed by Ph. Limbourg (1889), who added the sodium salt of cholalic acid to a mixture of peptone and pancreas extract. He inoculated this medium with dog-faeces and investigated certain of the chemical changes which took place. Leubuscher (1890) used pure bile as a medium for the growth of *B. anthracis*, *B. typhosus*, and other bacteria and found it a good culture medium. Corrado (1891) grew certain organisms in pure bile and came to the conclusion

I. that it behaves
  (*a*) indifferently towards *B. typhosus* and *B. pneumoniae*,
  (*b*) as a stimulant to the growth of *B. mallei*,
  (*c*) bactericidally towards *B. anthracis*, which dies after 48 hours in bile.

II. that only in the case of the anthrax bacillus was a weakening of virulence noted. After 18 hours sojourn in bile this bacillus became non-pathogenic to animals.

Fraenkel and Krause (1899) concluded that *B. typhosus* suffered at any rate no loss of virulence during a 24 hours exposure to the influence of bile. They cite Mosse and also Falck as having used bile media. Matzuschita (1902) used a decoction of liver in preparing an agar medium and found it the best solid medium for faeces bacteria. Conradi (1906) strongly advocated the use of pure bile as a help towards the isolation of *B. typhosus* from the blood in cases of typhoid fever. Meyerstein (1907) added the salts of bile to a nutrient medium and grew various organisms in it. His results led him to the conclusion that :

(1) *B. pyocyaneus* would grow well in a simple solution of bile salt,

(2) the same solution, with a small amount of nutrient substance added to it, formed a good medium for the growth of *B. coli*, and

(3) even the addition of large amounts of nutrient substances did not prevent it having an inhibitory action on the growth of *Staphylococcus pyogenes aureus.*

My first experiments were made in 1897 with a potato juice medium containing commercial bile salts; and these led to the suggestion in 1900 of bile salt lactose agar as a medium for *B. typhosus* and *B. coli communis.* The details of further experiments in which pure bile salts and bile acids were used were published in 1901 and in the same year Hill and I proposed bile salt glucose broth as a simple test for faecal contamination. A lactose broth had been previously used in our work for the Royal Commission on Sewage Disposal and glucose was substituted for the lactose merely so as to include such organisms as *B. enteritidis* (Gaertner). Of course, as stated by me (1904) in *Public Health*, if it is only a question of the presence of *B. coli communis* and its allies lactose broth is better than glucose broth.

The composition of the agar was as follows:—

Bile Salt	0·5%
Peptone	2·0%
Lactose	1·0%
Agar	1·5%
Tap Water	100 c.c.

and of the broth:

Bile Salt	0·5%
Peptone	2·0%
Glucose	0·5%
Litmus solution	q.s.
Tap Water	100 c.c.

On the agar, those organisms which decomposed the lactose produced acid in their immediate neighbourhood, and thus precipitated the bile salts with the consequent formation of a haze in the medium. This haze round the colonies differentiated them from those of non-lactose fermenters round which the medium remained quite clear.

Soon however it was observed that not every lactose-fermenting organism produced a haze when growing *on the surface* of the medium.

The reason for this will be apparent when Theobald Smith's work is referred to later on. At this time Grünbaum and Hume (1902) published their paper in which they proposed a modification of bile salt lactose agar. Their modification consists in the addition of 4 c.c. of $\frac{n}{1}$ NaHO to every litre of the agar and in the use of neutral-red as an indicator of the presence of acidity. They also say "the addition of Crystal Violet (1 in 100,000) permits of a striking double stain of the colonies." I had before this used neutral-red in bile salt broth for the "fluorescence test" but had not thought of it as an indicator. It seemed that the addition of this dye would do away with the difficulty occasioned by the absence of a haze round the surface colonies of some lactose fermenting organisms. It certainly acts very well as many colonies are pink and without a haze, but one still comes across surface colonies which, though colourless on neutral-red bile salt lactose agar, turn out to be colonies of lactose fermenting bacteria. The use of Crystal Violet is quite unnecessary as bile salt media alone inhibit most of the organisms found in air and soil (cf. MacConkey, 1900, p. 56) when the incubation temperature is 37° C. and over. The addition of 4 c.c. of $\frac{n}{1}$ NaOH per litre is to my mind a great disadvantage. The medium should be as neutral as possible to neutral-red, as otherwise organisms which attack the sugars, etc., only slowly and feebly may not be able to manufacture acid quickly enough and in sufficient quantity to overcome the alkalinity. Theobald Smith's observations are pertinent to this question. He says (1890) that in the absence of sugar both *B. coli* and the Hog Cholera bacillus are alkali producers. If a small quantity of sugar is present then, if the bacillus can act upon it, there is a rapid acid production and a slow alkali production. If the amount of acid is not too great it is neutralised by the alkali and the medium becomes alkaline. If the amount of acid is too great to be neutralised it tends to inhibit the growth of the bacillus. A similar result is obtained in the case of *B. typhosus*. Again in 1895 he states that more than 0·5% of glucose is harmful (cf. MacConkey, 1900, p. 48). The amount of acid produced is the same aerobically and anaerobically. There is a coincident production of alkali which is bound up with the growth of the organism, and which in the case of facultative anaerobes only goes on actively in the presence of oxygen. The acidity depends on the decomposition of sugar, etc.; the alkalinity, in the presence of oxygen, upon the growth of the organism. He states further that if too little sugar be present the alkali production masks the acid production unless oxygen be removed.

My own observations entirely confirm these statements. An inoculated tube very rarely remains neutral; it almost always becomes either acid or alkaline. It may become acid and remain so; the initial acidity may be followed by alkalinity; or the appearance of alkalinity may be the first perceptible change. I have seen a red surface colony of *B. coli communis* on a litmus lactose agar plate gradually lose its redness and in the course of time become distinctly blue.

It is obvious therefore that the medium should be as neutral as possible to the indicator used[1]. I have used both litmus and neutral-red and have come to the conclusion that the latter is to be preferred. It gives a lighter coloured medium and much better differentiation. Grünbaum and Hume's suggestion has been adopted by me, but not in its entirety. The added alkali has been omitted and the neutral-red accepted to the extent of using half the quantity mentioned in their formula.

The media are now prepared as follows. First a stock solution is made consisting of:—

Sod. Taurochol. (commercial from ox bile)	0·5%
Peptone (Witte)	2·0%
Water (distilled or tap)	100·0 c.c.

It is absolutely necessary to use bile salts which are neutral to neutral-red. Some samples are acid to this indicator and their use causes the finished medium to have a tint which makes the recognition of slight acidity somewhat troublesome. If litmus is preferred as an indicator it is not necessary to be so particular about the reaction of the bile salts. All the samples of bile salts I have tested have been alkaline to litmus. In this respect litmus has the advantage over neutral-red. Bile salts obtained from pig's bile are as far as my experience goes much more troublesome to use than those from ox bile. Calcium in the proportion of 0·03% of $CaCl_2$ seems beneficial to the growth of some of these organisms (cf. MacConkey, 1905, p. 336), and therefore this salt should be added when distilled water is used to make up the medium. Whenever possible I use tap water on this account.

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[1] The use of test tubes made of glass which yields an appreciable amount of alkali to the contained liquid may cause the medium to become too alkaline.

When a solution of bile salts is mixed with a solution of commercial peptone a precipitate is formed (cf. Maly and Emich, 1883). There may be some difficulty in getting rid of this. The best way to obtain a clear solution is to mix the ingredients together, steam them for 1—2 hours in a Koch's steamer, filter while hot and then allow to stand (24—48 hours if possible) until quite cold and sedimentation has occurred. Filtration through paper will then remove the precipitate and yield a clear liquid, which appears to keep indefinitely under proper conditions.

The various bile salt broths are prepared from this stock solution by adding glucose 0·5 %, lactose 1 %, cane sugar 1 %, dulcit 0·5 %, adonit 0·5 % or inulin 1 %, and neutral-red (1 % solution) 0·25 %, distributing into Durham's fermentation tubes and sterilising in the steamer for 15 minutes on each of three successive days. I have never experienced any disappointment from using a bile salt medium which had been steamed only twice, but it is best to be on the safe side and steam three times. Great care must be taken not to heat above 100° C. or for too long a time after the addition of the sugars, as otherwise the medium may be spoiled. The amount of dulcit and of adonit has been decreased on account of the great increase in the price of these substances.

Bile salt agar is made by dissolving 2 % of agar in the stock fluid either in the steamer or in the autoclave. The mixture is cleared with the white or the whole of an egg, filtered, neutral-red added in the same proportion as for broth, and distributed into flasks in quantities of 80 c.c., which is enough to make three plates of the usual size. When required for use the fermentable substance is added to the agar in the flask and the whole placed in a water bath or steamer. When melted the agar is poured into Petri dishes, allowed to solidify, and then dried in the incubator or warm room, the plate being placed upside down with the bottom detached and propped up on the edge of the cover. It is necessary that the surface of the agar should not be wet, as in that case the colonies will most likely form a confluent mass; nor should it be too dry as then the colonies are stunted in their growth. Inoculations are made by placing a loopful of a liquid culture or emulsion of the material to be examined on the centre of the agar in one plate and rubbing it over the surface with a bent glass rod; the same rod, without recharging, being used to inoculate the surface of the other two plates (Drigalski and Conradi's method). The plates are incubated upside down.

As regards the temperature of incubation 42° C. has of course a more selective action than one of 37° C., but the latter is quite good enough for ordinary purposes and is the temperature at which I have done all my work during the last four years. Therefore a special incubator is not necessary (cf. W. H. C. Forster, 1905).

Nor are anaerobic conditions of incubation absolutely essential. I have used them in the way of experiment to see if thereby any great advantage was to be gained, but I could not satisfy myself that the gain was sufficiently great to warrant the introduction of this method into routine work. From the observations of Theobald Smith, to which I have referred, it is obvious that the absence of oxygen may be of use in the case of organisms with fermentative powers of a weak and slowly acting character. One would therefore like to make use of anaerobic conditions, but the various forms of apparatus designed up to the present are so cumbersome that the trouble involved in their use outweighs the gain due to anaerobiosis. Besides, if an organism which ferments, say lactose, does give a colourless colony on bile salt lactose agar it is easy to subculture into a lactose broth tube in which, if freshly steamed, we have sufficiently anaerobic conditions to clear up any doubtful point.

Of course if one desires to compare with regard to the production of acidity the growth of an organism in a broth tube with the growth of the same organism on the surface of an agar plate anaerobic conditions are essential, as in the presence of oxygen one might get evidence of acid formation in the broth tube and none on the plate.

It will have been noted that the sugars, etc., are not added to the agar until just before use. The object of this procedure is to allow that kind of agar to be used which is most suitable to the case in question. By using a lactose agar the lactose fermenters, may be separated from the non-lactose fermenters. A mannit agar will pick out the mannit fermenters; a dulcit agar the dulcit fermenters, and so on. By combining two or more sugars, etc., in one agar we can exclude the organisms which attack these substances and leave only the colourless colonies for investigation. The use of a combination of several fermentable substances in one agar, suggested by me in 1905 (p. 335), has been adopted by Dr Houston (1907, p. 45).

With the idea of stimulating the growth of *B. typhosus* I have tried the effect of adding various substances to bile salt agar but without success. Urea, asparagin, nutrose, somatose, roborat, plasmon, and Nährstoff Heyden were without effect.

Soon after Loeffler published his method I investigated the effect of the addition of malachite green (Höchst 120) in varying proportions to bile salt agar and came to the conclusion that this medium could not be relied upon to give satisfactory results.

Encouraging results have been obtained by combining caffein (Roth's method) with bile salt agar when the amount of caffein was 0·33 %. But up to the present this combination has not proved satisfactory enough to induce me to recommend it for general use.

The method which has given me the best result in the isolation of lactose fermenters is as follows:

The material to be examined is inoculated, either diluted or undiluted, into bile salt lactose broth. In the case of a water, the amounts taken are 250 c.c., 100 c.c., 10 c.c., 1 c.c., 0·1 c.c. and 0·01 c.c.

Concentrated bile salt broth is added to the 250 c.c., and 100 c.c.; ordinary broth is added to the 10 c.c.; and the other quantities are added to the bile salt broth in tubes.

After 18—24 hours' incubation at 37° C. the highest dilution which shows any change is picked out and surface cultures are made from it on bile salt lactose agar. The unchanged tubes etc., together with the plates, are put in the incubator and examined next day. If any change in the direction of acidity has taken place in any of the tubes, plates are made from them.

If on examining the plates all the colonies appear alike, then only three colonies are subcultured; but if more than one kind of colony is present then two colonies of each variety are subcultured. I have repeatedly tried to distinguish one organism from another by the appearance of the colonies on bile salt neutral-red lactose agar but invariably without success. On the same plate an organism may give irregular filmy "coli-like" red colonies and also round raised "aerogenes-like" red colonies. The form of a colony on bile salt agar *is of no value in identifying a lactose fermenting organism.*

If bile salt agar plates inoculated with a mixture of lactose fermenters are left upside down at room temperature a peculiar change occurs in some of the colonies. They take on a mucoid character and increase so much in depth that portions of the growth may drop down on to the cover of the plate.

The best medium to use for making cultures from the colonies is slant agar; and the growth is rubbed over the whole of the surface of the medium. After 18—24 hours' incubation at 37° C. subcultures are made from the agar into the various media; a good loopful of culture being put into each tube. The sloped agar is used in order to get a sufficiently large quantity of growth and a large loop is used for the inoculations, because the mass of growth used for inoculation seems to have an effect in determining the rapidity of fermentation. By this means results are often obtained in 48 hours which otherwise might not be obtained under a week.

The media used are:—

1. *Glucose broth*—for Voges and Proskauer's reaction[1].

2. *Peptone water*—for indole.

This test should be performed after seven days' incubation; four days is not always enough even when using the Benzaldehyde reaction. If preferred, the peptone water may be omitted and the test performed upon an alcoholic extract of the agar growth (cf. Böhme, 1905).

3. *Litmus milk*—up to the present I have always made use of litmus milk. The experience thus gained has forced me to the conclusion that the litmus milk test is not necessary in the case of lactose fermenters.

4. *Gelatin*—I do not think much weight can be attached to the appearance of growths on gelatin of those organisms which grow on bile salt media. I use gelatin simply for the purpose of observing the presence or absence of liquefaction. Liquefaction may take place rapidly or slowly. Some organisms take 6—9 months to liquefy half an inch of gelatin (e.g. *B. oxytocus perniciosus*). By appropriate means the time may be shortened somewhat (MacConkey, 1906), but even then liquefaction may require a month to be complete. Retardation of solidification after being melted may be noticeable 2—3 weeks before the completion of liquefaction. Now in routine work it is quite impossible to wait longer than about two weeks. So in practice we are at present forced to class with the non-liquefiers any organism which does not liquefy gelatin in a fortnight.

5. *Bile salt broths*—containing respectively lactose, cane-sugar, dulcit, adonit and inulin[2].

After inoculation, the tubes containing these media are kept under observation as long as there is no change in the reaction of the medium[3] As long as the medium remains neutral there is a possibility of the production of acid, but when once the reaction of alkalinity is visible in the inner tube the tubes may be discarded as acid production will not occur subsequently.

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[1] Recently I have isolated organisms which sometimes gave this reaction and sometimes did not do so. It may therefore be necessary to modify one's opinion with regard to this test.

[2] Inosit also has a value for differentiation purposes.

[3] A week or 10 days is long enough in practice.

Gas may not be apparent for two or three days after the medium has turned acid.

Examinations are also made as to the presence or absence of motility and as to whether the organisms are Gram-positive or Gram-negative.

If the liquid media are inoculated in the morning, say between 10 and 11 A.M., they may be examined for motility the same afternoon between 4 and 5 P.M. It is not necessary to have visible growth for this purpose. A tube in which no growth is apparent to the naked eye may be fairly full of bacilli. To my mind the best idea of motility is gained by using a low power objective (half an inch), an eye-piece magnifying 8—10 times and dark ground illumination. This arrangement gives excellent results also in the case of agglutination reactions.

In the early stages of working with bile salt agar the results were somewhat conflicting and I gained the impression that this medium exercised a certain amount of inhibitory action on the growth of *B. coli communis* and *B. typhosus*.

As experience increased however it became apparent that, while some inhibitory effect might be exerted on organisms which had been grown on artificial media for a long time, freshly isolated organisms grew well.

As regards bile salt broth: Forster (1905) tested it against Drigalski and Conradi's nutrose agar. He used dilutions of a very thin emulsion of horse dung in sterile water for the inoculating material, with the following result:—

Nutrose agar aerobically at 37° C. for 24 hours.		MacConkey's medium anaerobically at 42° C. for 24 hours.	
Tube 1=0·1 c.c.	(growth)	Tube 1=0·1 c.c.	(growth)
2=0·01 c.c.	,,	2=0·01 c.c.	,,
3=0·001 c.c.	,,	3=0·001 c.c.	,,
4=0·0001 c.c.	,,	4=0·0001 c.c.	,,
5=0·00001 c.c.	,,	5=0·00001 c.c.	,,
6=0·000001 c.c.	(sterile)	6=0·000001 c.c.	(sterile)
7=0·0000001 c.c.	,,	7=0·0000001 c.c.	,,
8=0·00000001 c.c.	,,	8=0·00000001 c.c.	,,

By subculture *B. coli* was isolated from tube 5 of both series.

Thresh and Sowden (1904) compared phenol broth with bile salt broth in the examination of waters. They found that they obtained a positive reaction in bile salt broth from a quantity of water which yielded no growth in phenol broth.

The following are experiments made by myself.

(I) A 1 in 100 emulsion of horse and calf faeces was put into a sterile Berkefeld filter (F 3) which was suspended in a jar of tap water. The water in the jar was changed every day. At the end of 12 weeks the contents of the filter were thoroughly mixed and a small quantity removed for examination. Dilutions were made up to 1 in 1000 millions. One cubic centimetre of each dilution from 1/1000 upwards was put into bile salt lactose broth and the same quantity also into ordinary alkaline nutrient bouillon. The tubes were incubated at 37° C. with the following result:—

Bile salt lactose bouillon 24 hours at 37° C.		Alkaline nutrient bouillon 24 hours at 37° C.	
Tube 1=1/1,000	A+G	Tube 1=1/1,000	Growth
2=1/10,000	A+G	2=1/10,000	,,
3=1/100,000	A+G	3=1/100,000	,,
4=1/1,000,000	No growth	4=1/1,000,000	No growth
5=1/10,000,000	,,	5=1/10,000,000	,,
6=1/100,000,000	,,	6=1/100,000,000	,,
7=1/1,000,000,000	,,	7=1/100,000,000	,,
48 hours at 37° C.		48 hours at 37° C.	
Tube 4	Growth, no acid	Tube 4	Growth
5	No growth	5	No growth
6	,,	6	,,
7	,,	7	,,

A=acid, G=gas.

Further investigation showed that there were lactose fermenters present in both kinds of broth in all dilutions up to 1/100,000 but not beyond; and that organisms were present in both media in the 1/1,000,000 dilution but there was no growth in any higher dilution.

In this case bile salt lactose broth proved just as good a medium as ordinary alkaline nutrient bouillon.

(II) On another occasion a similar experiment was performed with two other filters (F 4 and F 5) each containing a 1 in 100 emulsion of human faeces. After two months in tap water the contents were examined in exactly the same manner as in the case of F 3. The results were:—

F 4.

Bile salt lactose broth 24 hours at 37° C.		Alkaline nutrient bouillon 24 hours at 37° C.	
Tube 1=1/1,000	A+G	Tube 1=1/1,000	Growth
2=1/10,000	A+G	2=1/10,000	,,
3=1/100,000	A	3=1/100,000	,,
4=1/1,000,000	A	4=1/1,000,000	,,
5=1/10,000,000	Nil	5=1/10,000,000	Nil
6=1/100,000,000	,,	6=1/100,000,000	,,
7=1/1,000,000,000	,,	7=1/1,000,000,000	,,
48 hours at 37° C.		48 hours at 37° C.	
Tube 3=1/100,000	A+G	Tube 3=1/100,000	Growth
4=1/1,000,000	A	4=1/1,000,000	,,
5=1/10,000,000	? growth	5=1/10,000,000	,,
6=1/100,000,000	,,	6 – 1/100,000,000	? Growth
7=1/1,000,000,000	,,	7=1/1,000,000,000	,,

A=acid, G=gas.

Further investigation proved that there was growth in all tubes up to 1/10,000,000 but not beyond; and that lactose fermenters were present only up to 1/1,000,000.

F 5.

Bile salt lactose broth 24 hours at 37° C.		Alkaline bouillon 24 hours at 37° C.	
Tube 1=1/1,000	A+G	Tube 1=1/1,000	Growth
2=1/10,000	A+G	2=1/10,000	,,
3=1/100,000	A+G	3=1/100,000	,,
4=1/1,000,000	A+G	4=1/1,000,000	,,
5=1/10,000,000	A	5=1/10,000,000	,,
6=1/100,000,000	A	6=1/100,000,000	Nil
7=1/1,000,000,000	Nil	7=1/1,000,000,000	,,
48 hours at 37° C.		48 hours at 37° C.	
Tube 5=1/10,000,000	A + fluorescence	Tube 5=1/10,000,000	Growth
6=1/100,000,000	A+G	6=1/100,000,000	,,
7=1/1,000,000,000	?	7=1/1,000,000,000	?

A=acid, G=gas.

Subsequent inoculations showed growth in bile salt lactose broth up to 1/1,000,000,000 and in alkaline bouillon up to 1/100,000,000; but lactose fermenters were present only up to 1/100,000,000 in bile salt broth and up to 1/10,000,000 in alkaline bouillon.

The results of the examination of the contents of filters 4 and 5 after a sojourn of two months in tap water confirm previous experience and prove that bile salt broth is quite as good as any other medium for water analysis.

Bile salt in a proportion of 0·5 % has no inhibiting effect upon *B. typhosus, B. coli communis* and allied organisms. As a matter of fact these bacilli will multiply in a broth containing a much larger percentage of bile salt. I have kept *B. typhosus,* etc. in 5 % bile salt broth at room temperature for six weeks, and at the end of that time found them alive and capable of giving an abundant growth when re-inoculated on to agar. Of course there were no fermentable substances in the bile salt broth of this particular strength as the production of acid would have vitiated the experiment.

I have also tried the effect of increasing the quantity of bile salt in bile salt agar. The percentages used were 4, 5, 6, 7, 8 and 9 %, and some 30 known organisms were tested. The results may be tabulated thus:—

B. prodigiosus *B. mesentericus fuscus* *B. mesentericus vulgatus*	did not grow.
B. cavicida (Brieger)	Growth in 4 % in 48 hrs. „ „ 6 % „ 4 days.
B. oxytocus perniciosus	,, ,, ,, ,, ,,
B. lactis aerogenes *B. capsulatus* (Pfeiffer) *B. cloacae*	Poor growth on 9 % in 48 hrs.
B. typhosus *B. coli communis* (Escherich) *B. acidi lactici* (Hüppe) *B. neapolitanus* *B. pneumoniae* (Friedländer) *B. levans* *B. enteritidis* (Gaertner) *B. paratyphosus* etc., etc.	Appeared to be unaffected except that some members of the Gaertner group (e.g. Hog Cholera, Arkansas—Smith) grew slowly during the first 24 hours.

The following organisms will not grow on ordinary bile salt media.

B. anthracis,
B. anthracoides,
B. diphtheriae,
B. xerosis,
B. subtilis,
M. tetragenus,

B. faecalis alkaligenes grows well on bile salt media that have been made strongly alkaline; but not on the ordinary media.

Streptococci vary very much, some grow well but slowly, others scarcely at all.

Summary.

Bile salt media have been in use since 1900, and during the seven years which have elapsed it has been shown that *B. typhosus*, *B. enteritidis* (Gaertner), *B. coli communis* and similar organisms grow on these media just as well as on any other nutrient media. At 37° C. the growth of most of the organisms of the air and soil is inhibited by bile salt media.

These media can therefore be used with confidence for the isolation of *B. typhosus* and other intestinal organisms.

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ON THE RELATIONSHIP BETWEEN *BACILLUS PESTIS* AND *BACILLUS PSEUDOTUBERCULOSIS RODENTIUM* (PFEIFFER).

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The *Bacillus pseudotuberculosis rodentium* (Pfeiffer) is the organism which morphologically and culturally most closely resembles *Bacillus pestis.* Galli-Valerio (1903) concluded that these bacilli resemble each other very closely in most respects but that *B. pseudotuberculosis* coagulates milk while the *B. pestis* does not; and while the *B. pseudotuberculosis* is not pathogenic for rats, it is extremely so for guinea-pigs, which succumb to cutaneous, nasal and conjunctival inoculations.

Zlatogoroff (1904) from a comparative study of 2 strains of the *B. pseudotuberculosis* and 22 strains of *B. pestis* (2 from rats and 20 from men) expresses the opinion that morphologically and culturally these organisms are practically identical; though *B. pseudotuberculosis* does not show such extensive pleomorphism as *B. pestis* and is non-virulent for rats and pigeons and only slightly so for white mice. The agglutination titre of the pest serum he tested did not exceed 1 in 500 and "clumping" took place quite as well in the case of *B. pseudotuberculosis* as in that of *B. pestis.* He obtained precipitin reactions in pest serum only with pest filtrates. Pest serum did not protect against infection with the *B. pseudotuberculosis* and *vice versa.* He failed entirely in his attempts to produce cross immunisation.

I have had at my disposal for study 11 strains of *B. pestis* and 7 of *B. pseudotuberculosis rodentium* (Pfeiffer) of various origins. The convention ΨT.R. is used to designate the pseudotubercle bacillus.

When grown on agar or gelatine or in neutral bouillon no *constant* difference could be made out as regards the appearance of the growths or the morphology and staining reactions of the bacilli. The growth of

B. pestis on agar is usually found to be of a stringy consistency when touched with a needle but this is not a constant feature (cf. Shibayama, 1905). Not infrequently it is short and granular like that of the Bac. ΨT.R. On the other hand none of my strains of ΨT.R. ever gave a viscid growth; and they almost always grew more abundantly than *B. pestis*.

In litmus milk all the 18 cultures both of pest and pseudotuberculosis produced no change for three to four days at 35° C. Then in the case of the ΨT.R. cultures the medium began to turn alkaline and finally became deep blue. The pest cultures were kept at 35° C. for about a week and then at room temperature for five weeks. At the end of this time there was no change apparent in the medium. On subculture an abundant growth of pest was obtained. In bile salt media containing various fermentable substances the strains of each gave the same reactions, and in this connection it is worthy of note that the ages of these strains varied from a few weeks to ten years. Acid but no gas is produced from glucose, mannite, laevulose, galactose and dextrin, while lactose, cane sugar, raffinose, sorbit, dulcit, adonit, inulin, amygdalin and α-methyl glucoside are unaffected (cf. MacConkey, 1905, p. 350). Vourloud (1907) obtained slightly different fermentation reactions, but he used ordinary nutrient agar and a trace of muscle sugar may have been present.

All these races were inoculated on to salt agar (NaCl 3 %). At the end of 24 hours' incubation at 30° C. there was nothing to choose between them in the appearance of the growths. The growth of ΨT.R. might have been slightly more abundant but that was all. Microscopically one could pick out a strain of pest which differed somewhat from some one particular strain of ΨT.R.; but taking all the cultures together there was no bacillary form appearing among the films of pest which was not represented among those of ΨT.R. also. After 48 hours' growth at 30° C. the majority of the cultures of ΨT.R. showed a more abundant growth than the majority of the cultures of pest, but still there were some of the cultures of ΨT.R. which had only grown slightly, while one culture of pest (10 years old) had grown more vigorously than any of the strains of ΨT.R. Microscopically the relative appearances were the same as at 24 hours. The general impression gathered from a microscopical examination of all these cultures was that the yeast-like involution forms were not so abundant in the cultures of ΨT.R., though one culture of ΨT.R. was full of such forms, in fact fuller than any of the pest cultures.

We find then culturally only two points of difference between these bacilli, namely the frequently viscid character of the growth of pest on agar and the development of an alkaline reaction in tubes of litmus milk inoculated with the Bac. ΨT.R. These results are quite in accordance with those of Zlatogoroff, and as far as one can form an opinion from cultural characters one is constrained to recognise a close resemblance between these bacilli.

The results of precipitin tests emphasize this relationship. On the addition of pest filtrate to pest serum a cloudiness appeared almost at once and an abundant precipitate was deposited at the end of three hours at 37° C. When ΨT.R. filtrate was added to pest serum these changes did not take place so rapidly. After three hours at 37° C. there was a distinct precipitate suspended in the serum but no deposit at the bottom of the tube. After three hours at 37° C. the tubes were placed in the cold room overnight. The precipitate caused by ΨT.R. serum was not completely deposited at the end of 24 hours. On the other hand the pest filtrate precipitate was completely deposited and the supernatant fluid was quite clear.

The filtrates used were those employed to immunise guinea-pigs in the experiments on cross-immunisation detailed later.

Cross-immunisation.

Guinea-pigs are so very susceptible to infection by the Bac. ΨT.R. that it is not to be wondered at if, in trying to immunise them with this bacillus, the mortality is considerable.

It is necessary to proceed very slowly with the process of immunisation and a month is a good interval to place between the injections. If the inoculations are not properly spaced the mortality is too high. Even when one proceeds with the greatest care one cannot count upon keeping all the animals alive until the test inoculation.

This state of affairs obtains also in the case of rats, though rats are said to be immune to ΨT.R. It is true that they do not succumb to an acute disease but they do not all remain in as good health as before the inoculations. They waste and seem to become more susceptible to the attacks of other organisms. For instance in one experiment 12 white rats received killed cultures of the Bac. ΨT.R. mixed with their food, about 9 c.c. of a 24-hour broth culture each day. Ten of these 12 rats died and from five of these ten I isolated a bacillus belonging to the group of the *B. enteritidis* (Gaertner). During this time there was not a single death among our stock rats.

The experiments detailed below show that animals which have withstood the process of immunisation with the Bac. ΨT.R. are in most cases insusceptible to subsequent infection with pest.

Experiment I.

Four guinea-pigs were inoculated subcutaneously first with killed and then with living cultures of the Bac. ΨT.R. Two of these animals died after the second dose. The remaining two received a third injection and then two months later were inoculated subcutaneously with pest, one control being infected at the same time. The pest inoculations were performed by Captain S. R. Douglas, I.M.S. The control animal died of pest on the fifth day while the other animals showed no signs of illness. Two months later they were killed and examined. One showed no signs of disease whatever. In the other there was a caseous inguinal gland from which no growth could be obtained. A film made from the caseous material showed appearances which might have been bacilli.

Experiment II.

In this experiment two series of animals were used.

Series I. Twelve guinea-pigs were inoculated subcutaneously with a quantity of killed emulsion equal to $\frac{1}{40}$ of an agar culture of Bac. ΨT.R. Subsequently it was found that five does were pregnant. These were not used any more. Thirty-three days after the first injection a second inoculation of killed emulsion was given. This dose equalled $\frac{1}{10}$ of an agar culture. Thirty-seven days later each pig received killed emulsion equal to $\frac{1}{5}$ of an agar culture. Thirty-eight days later 1 c.c. of a living horse serum bouillon culture was given. Three pigs died after this dose. Forty-six days later the remaining four animals were inoculated with pest and found to be immune.

Series II. Twelve guinea-pigs were inoculated subcutaneously with killed emulsion of Bac. ΨT.R., the first six receiving a dose equal to $\frac{1}{20}$ and the remainder a dose equal to $\frac{1}{10}$ of a 2-day agar culture. One of the second six died in three weeks. Thirty-eight days after the first injection each animal received subcutaneously 1 c.c. of a 5-day horse serum bouillon living culture of the Bac. ΨT.R. Forty-six days later they received a test inoculation of pest at the same time as the animals in Series I and 10 controls.

The animals in Series II, like those in Series I, were found to be immune.

Of the controls seven died within a week and the *B. pestis* was isolated from every one. The remaining three were alive and well seven weeks later.

The result may be summarised thus:

Controls (10) mortality 70 %.
Immunised (15) mortality 0 %.

Experiment III. *To test the duration of the immunity.*

Ten guinea-pigs were inoculated subcutaneously with an emulsion of Bac. ΨT.R. which had been killed by heat. Each animal received an amount of emulsion equal to $\frac{1}{20}$ of an agar tube.

Another series of 10 animals was treated in precisely the same manner except that an emulsion of another strain of Bac. ΨT.R. was used.

Four of the first series and one of the second series died during the following 11 days.

Thirty-two days after the first injection a second dose was given subcutaneously, the quantity being equal to $\frac{1}{10}$ of an agar tube. A pig of the second series died after this inoculation.

After an interval of twenty-two days the animals received a third injection—the dose being 1 c.c. of a 24-hour living broth culture.

The animals were then kept for 204 days, i.e. nearly seven months, and were then given a test inoculation of pest; seven controls being inoculated at the same time.

The result was that six of the seven controls died of pest within eight days, three of the second series of immunised animals died on the seventh, eleventh, and twelfth days respectively, while one control and 11 immunised pigs remained alive and healthy.

Summarised result:

Controls (7) mortality 85·7 %.
Immunised (14) mortality 11·5 %.

It would appear then that in the majority of cases the immunity lasts at least six months.

Experiment IV. *To ascertain whether protection can be conferred by means of filtrates from cultures of ΨT.R. bacilli.*

A Roux bottle of nutrient agar was inoculated with ΨT.R. and incubated for three days at 37° C., when the growth was washed off the agar and emulsified in 200 c.c. of NaCl (0·85 %) solution. The emulsion was heated in a flask for an hour at 60° C., a few drops of chloroform added, the flask plugged with a rubber cork and autolysis allowed to proceed for eight days at 37° C. The contents of the flask were then filtered through a Berkefeld filter, thus giving "ΨT.R. filtrate."

An exactly similar procedure was carried out with a strain of pest, and "pest-filtrate" obtained.

Ten guinea-pigs were inoculated subcutaneously with 1 c.c. of pest filtrate and another 10 pigs with 1 c.c. of ΨT.R. filtrate. One pest pig and two ΨT.R. pigs died five days later.

After an interval of 14 days all the animals were re-inoculated, the dose being 2 c.c. of filtrate. Two pigs of each series died during the subsequent three weeks.

One month after the second injection the animals received each 5 c.c. of filtrate.

Thirty-seven days later two pest pigs, two ΨT.R. pigs and two controls received a test inoculation of pest.

The result was that both controls died of pest within eight days, one pest pig died on the tenth day, and the remaining pest pig and both the ΨT.R. pigs remained alive and well.

The remaining animals were kept to be tested later.

At the end of four months it was found that the virulence of the pest bacillus used for testing had greatly diminished and that a test could not be carried out. So each pig received 1 c.c. of filtrate subcutaneously to keep up the immunity.

Four months after this injection these animals and six controls received a test inoculation of pest.

As a result four out of five pest pigs, one out of three ΨT.R. pigs and five out of six controls died of pest.

The results of both parts of this experiment may be taken together and summarised thus:

Controls (8) mortality 87·5 %.
Pest immunised (7) mortality 71·5 %.
ΨT.R. immunised (5) mortality 20 %.

These results show that immunity to pest can be conferred by means of filtrates of cultures of the ΨT.R. bacillus and that this immunity lasts several months.

So far the experiments had been confined to guinea-pigs, animals which are acknowledged to be very susceptible both to pest and to ΨT.R. The question then naturally arose as to whether rats could be similarly protected by inoculations of the bacillus ΨT.R., an organism to which they are more or less immune.

Experiment V.

Two series of 10 rats each were inoculated subcutaneously with 0·5 c.c. of a living 24-hour broth culture of the Bac. ΨT.R., a different strain being used for each series. Seven weeks later they received 1 c.c. of a 24-hour living broth culture subcutaneously.

Seven animals died during immunisation.

One hundred and sixty (160) days after the second immunising injection Dr C. J. Martin kindly tested these rats with pest. Nine of the one series, four of the other series and five controls were inoculated at the same time. The result was that all the controls were dead of pest on the fourth day. Of the immunised animals three died on the fourth day, one on the fifth day and one on the seventh day. The remaining eight remained alive and healthy.

Thus of the

Controls (5) mortality 100 °/o,
Protected (13) mortality 38·5 °/o,

when tested five months after the last immunising injection.

Summary.

(1) Morphologically and culturally the *Bacillus pseudotuberculosis rodentium* (Pfeiffer) bears a strong resemblance to *B. pestis.*

(2) The filtrate from an autolysed agar culture of *B. pseudotuberculosis rodentium* (Pfeiffer) and a similar filtrate from a *B. pestis* culture both gave a precipitin reaction with pest serum.

(3) It has been found possible to immunise both guinea-pigs and rats against plague by means of inoculations of cultures of *B. pseudotuberculosis rodentium* (Pfeiffer), and this immunity lasted in many cases at least six months.

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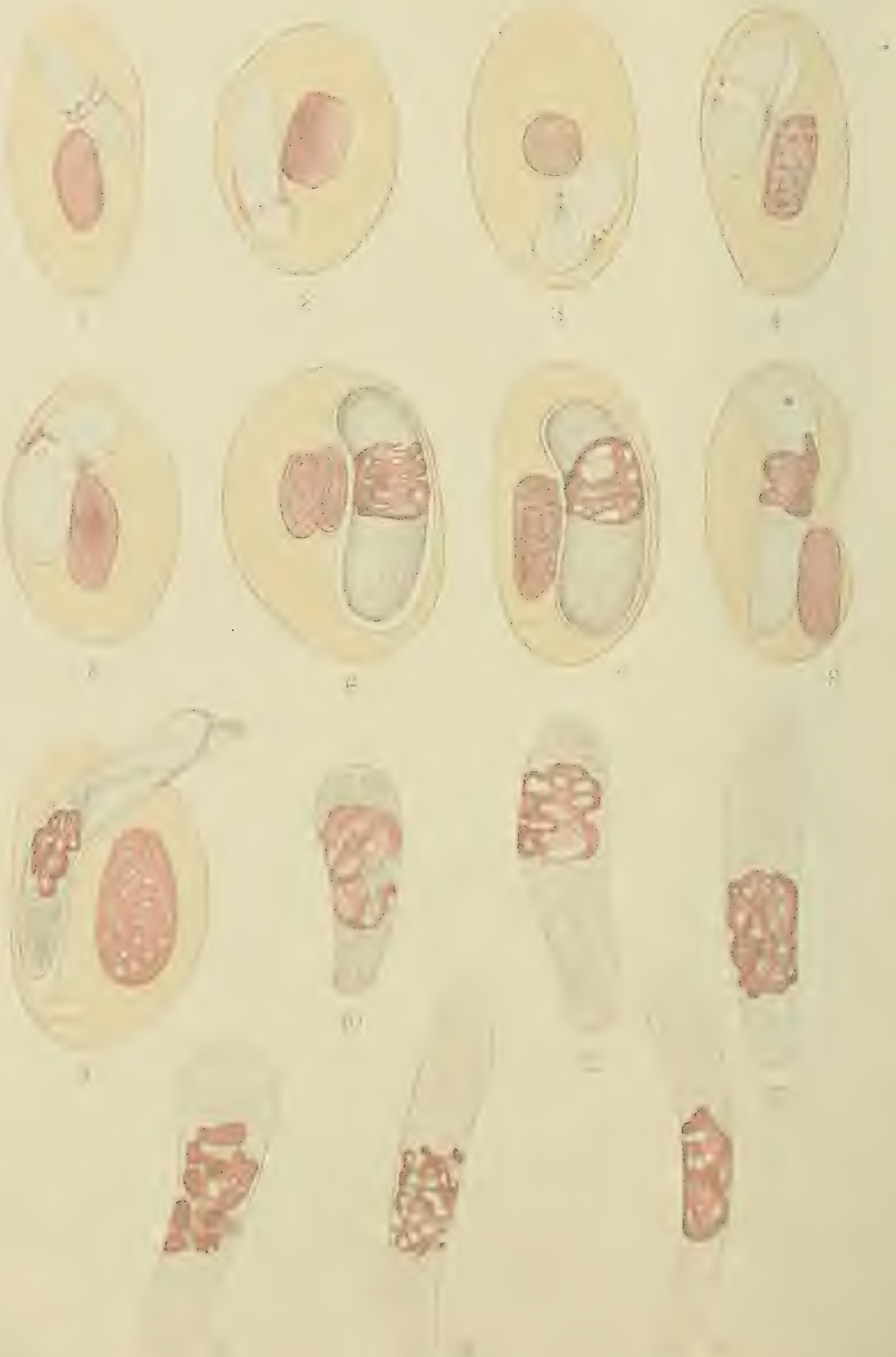
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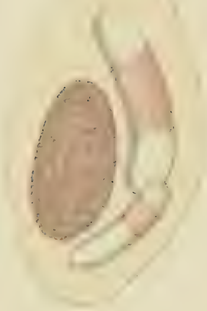

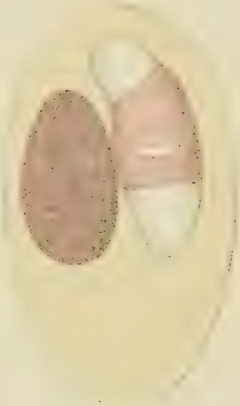

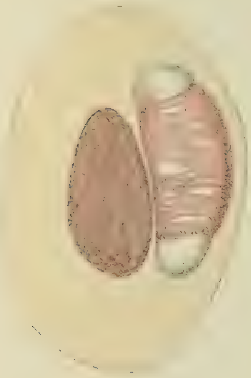

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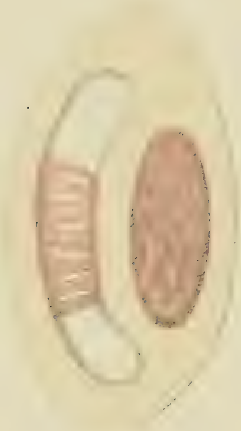

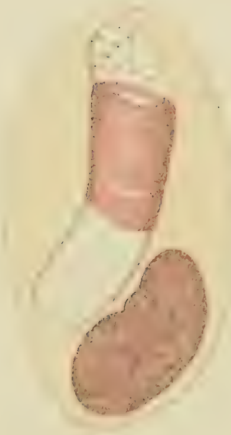

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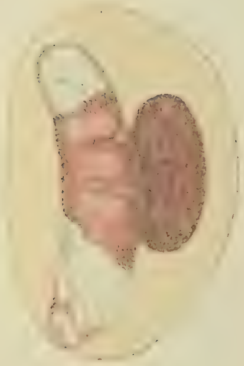

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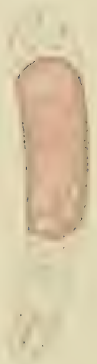

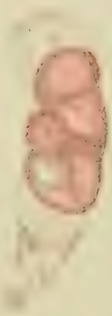

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On a Hæmogregarine from the Blood of a Himalayan Lizard (*Agama tuberculata*). By E. A. MINCHIN, M.A., F.Z.S.

(Plates LV. & LVI.)

The material upon which the following description is based consists of four slides, bearing smears of the blood of the common rock-lizard of the Himalayas, which were prepared and sent to me from Kasauli by Lt.-Col. F. Wyville-Thomson, I.M.S., to whom my best thanks are due for his kindness in sending me the specimens and entrusting me with the description of them. I shall refer to the slides by the letters A–D. An examination of the slides shows at once that they fall into two pairs, A and B being one pair, C and D the other. While the parasites are very similar, both as regards structure and occurrence, in both the slides of each pair, those of one pair are so different from those found in the other pair of slides that they might easily be taken for distinct species of parasites. At this distance from the habitat of the host and its parasite, and with limited material at my disposal, I can only record these differences, and cannot attempt to explain them.

Preparation of the Slides.—Slide A, alone of the four, was sent to me unfixed; my assistant Dr. Woodcock fixed it in the usual way with methyl alcohol, and stained it for me with Giemsa's modification of the Romanowsky stain, subsequently differentiated with Unna's tannin-orange solution. The other three slides were sent to me already fixed and stained with Leishman's stain, by Lt.-Col. Wyville-Thomson. It is important to note, therefore, that, for aught I know to the contrary, slide B was prepared in the same manner as slides C and D, but in a different way from slide A; so that the resemblances and differences, presently to be described, exhibited by the four slides, cannot be ascribed to like or unlike methods of preparation in each case. The only differences that can be attributed to the action of the stains used are, that in slide A the ground-colour of the red blood-corpuscles is lighter and more yellowish in tint, in B, C, and D darker and more greyish, and that in slide A the nuclei of the red blood-corpuscles and of the parasites scarcely differ in tint, but in B, C, and D the nuclei of the red blood-corpuscles have a distinctly purplish tinge, while the nuclei of the parasites show a more pronounced red colour.

Description of the Parasite.—In all the slides there are to be found both intra-corpuscular forms and free forms, so-called vermicules. The free forms are sufficiently similar to the largest intra-corpuscular forms to warrant the assumption that they have escaped from the corpuscle, and I have twice found parasites apparently in the act of escaping from the corpuscle (Pl. LV. fig. 9).

The intra-corpuscular forms, in their youngest stages, are placed at the side of the nucleus of the blood-corpuscle, which is then normal in appearance, with the nucleus occupying its proper central position. When the parasite is full-grown, however, the nucleus of the corpuscle is more or less pushed to one side, and may become somewhat irregular in outline; but in no case have I seen the corpuscular nucleus at all broken up, as is known to occur in many cases of reptilian hæmogregarines, hence classed by some authors as a distinct genus *Karyolysus*. I have observed, however, a peculiar relation between the shape of the parasite and the displacement of the corpuscular nucleus. The intra-corpuscular parasite is always distinctly sausage-shaped, and slightly bowed in the plane of the corpuscle. It is reasonable to attribute this curvature to the fact that the parasite, being situated to the side of the corpuscular nucleus, accommodates itself to the space in which it lies (compare figs. 5 & 6, Pl. LV.). We may speak of this as the normal curve of the parasite. But in a few cases we find the curve of the parasite entirely reversed, and its convexity turned towards the nucleus of the corpuscle (Pl. LV. fig. 8, Pl. LVI. figs. 20, 21). In such cases two points are noticeable: first, that the parasites resemble the free forms very closely; secondly, that the corpuscular nucleus is much more displaced than usual, being sometimes pressed quite against the side of the corpuscle (figs. 8 & 20). This point has also been noticed by Lt.-Col. Wyville-Thomson, who has sent me four sketches of the parasite, representing one free and three intra-corpuscular forms: two of the latter show the normal curvature of the parasite, like my fig. 19 on Pl. LVI.; the third, however, shows a reversed curve, with the nucleus of the corpuscle pushed far to the side, as in my fig. 20. I think we shall not be far wrong in attributing these cases of reversed curvature to the commencing activity of a parasite about to become free from the corpuscle, in which the movements of the contractile body not only alter its curvature, but also may have the effect of forcing the nucleus of the corpuscle away to one side.

I will now proceed to describe the structure of the parasite in more detail. Beginning with slides A and B, we find forms of the parasite which may be classified as young intra-corpuscular forms, full-grown intra-corpuscular forms, and free forms or vermicules.

The young intra-corpuscular forms (Pl. LV. figs. 1–5) vary in length from about $\frac{1}{2}$–$\frac{2}{3}$ of the blood-corpuscle; *i.e.*, from about 9–11 μ in length[*]. They were remarkable in two points: first, their very clear cytoplasm, hyaline and free from granulations, so that they often have the appearance of a space in the blood-corpuscle; and, secondly, their delicate nucleus, which appears to consist of faintly-staining granules and strands of chromatin, forming, so to speak, a band round the waist of the parasite; in many cases, however, it is an " Empire " waist, placed much nearer one end of the body than the other. As I have laboured to reproduce the appearance of the objects in my illustrations, I need not enter into longer descriptions. While, as is shown, some parasites have slightly more chromatin than others, there is nothing that can be said to be in the least transitional to the forms next to be described.

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[*] Since all the figures which accompany this memoir are drawn to a magnification of 2000 linear, it follows that a length of 2 mm. in the drawings corresponds to an actual length of 1 μ in the objects.

The full-grown intra-corpuscular parasites are at least $\frac{3}{4}$ the length of the blood-corpuscle, *i. e.* about 15–17 μ in length by about 5 μ in breadth. They contrast sharply with the young forms in the characters both of the body and nucleus, but especially the latter (Pl. LV. figs. 6–8). The body appears distinctly contoured and shaded, standing well off from the blood-corpuscle; it sometimes lies in a distinct clear space (figs. 6 & 7), but I could not make out anything of the nature of a capsule surrounding it. The cytoplasm is finely granular and takes a distinct bluish stain; only in one instance did I observe in the cytoplasm what appeared to be a grain of chromatin distinct from the nucleus, in a parasite which appeared to be ripe for escape from the corpuscle (fig. 8). But the nucleus is the most remarkable feature of the parasite at this stage, as compared with the forms described in the preceding paragraph; it is exceedingly rich in chromatin, which forms a deeply staining mass of irregularly spongy texture, occupying the middle region of the body for practically its whole width and nearly one-third of its length. Here, again, I must leave my illustrations to speak for themselves; I think they make the contrast between the young and old forms of the parasite sufficiently plain.

Of the free vermicules, it can be said that they resemble closely the full-grown intra-corpuscular forms. Their principal variations of form and structure are shown on Pl. LV. figs. 10–15; we can distinguish in a general way stumpy forms (fig. 10), medium forms (figs. 11–13), and long forms (figs. 14, 15), the last-named being by far the most abundant. One end of the body is always slightly clubbed, and the nucleus may be nearer to the stouter end, or to the narrower end, or to the middle of the body. The nucleus shows the same spongy structure described above for the intra-corpuscular parasites; it may vary in texture from a coarse to a more finely-knitted texture. Only in one case did I observe a tendency for the chromatin to take the form of more or less distinct masses (fig. 13).

I have figured (Pl. LV. fig. 9) one of the two cases in which I found the vermicule in the act, apparently, of escaping from the corpuscle. But even after examining the preparation by the aid of different objectives and various methods of illumination, I was not able to make up my mind exactly as to how the appearances seen should be interpreted in some points. My impression is that the projecting extremity of the parasite is rounded off, and that the conical pointed end seen in my figure represents something of the nature of a shrivelled sheath pushed out by the parasite. I could not, however, see any distinct line of separation between what I regard as sheath and as body, and it may be that the parasite, at the moment of leaving the corpuscle, has the anterior end irregular in shape. I have tried to depict the object as I saw it, and I must leave the matter there. The other example of this stage which I found was too much obscured by neighbouring blood-corpuscles to be made out clearly.

To complete my study of these slides, I made some counts of the different forms occurring in each. This was done with the help of the mechanical stage, going methodically over a part of the smear, so as to make sure that the same forms were not seen twice, and counting all the forms met with. The following is the result for slides A and B :—

	Young intra-corpuscular forms (figs. 1–5).	Full-grown intra-corpuscular forms (figs. 6–8).	Vermicules.		
			Long (fig. 15).	Medium (fig. 11).	Stumpy (fig. 10).
Slide A	14	5	...	93	...
Slide B	24	28	60	10	11

To these figures must be added the two parasites in the condition of fig. 9, found on slide B. It is seen that on both slides the free vermicules greatly preponderate, though this is less marked on slide B than on slide A ; and the more detailed count made of slide B shows that long forms of the vermicules are greatly in excess of shorter forms.

The other two slides, C and D, show a very different state of things. In the first place, free vermicules are comparatively rare, and young forms, poor in chromatin, like those described above, are not found at all. The majority of the parasites met with are intra-corpuscular forms similar to that depicted in fig. 19, on Pl. LVI. The parasite has the form of an elongated sausage, slender and drawn out, in very marked contrast to the stout forms already described (compare figs. 6 and 7); the normal curve is concave on the side towards the corpuscular nucleus. The cytoplasm of the body is usually clear and free from coarse granulations. But the most remarkable feature of the parasite is its nucleus, which forms a band or zone at the middle of the body, equal in width to nearly half the length of the body. The chromatin is arranged in the form of transverse strands, which seem to wrap round the body in this region ; between the strands of chromatin clear spaces are seen, in some specimens abundantly, while in other cases the texture may be closer and the structure more compact. The strands of chromatin often appear to bulge beyond the contour of the parasite, a point which Lt.-Col. Wyville-Thomson has remarked upon in his letter to me, and has depicted in the accompanying pencil-sketches. Nothing could be more different than the appearance of the nucleus of the parasite in these two slides C and D and that seen in slides A and B already described; I think my figures show this point with sufficient clearness.

Besides the slender elongated forms just described, there are found also (but less commonly) short, stumpy intra-corpuscular parasites (Pl. LVI. figs. 17, 18)—not in the least comparable, however, to the young forms found on slides A and B. They give me the impression, rightly or wrongly, of being dwarfed, stunted forms, which for some reason have not attained their full growth. Their nucleus is as large as, or even larger than, that of the long forms, and has the same type of structure, with the fenestrations even more apparent in many cases; it occupies at least half, sometimes much more than half, the length of the body, leaving two clear poles free from chromatin at either end.

A third type of intra-corpuscular parasite, found in a few cases (figs. 20, 21), was also stouter than the more elongated forms, and noteworthy for the presence of red-staining granules, apparently chromidia, in the cytoplasm outside the nucleus. Since these forms resemble, as will be shown, the free vermicules in this feature—and since they frequently occur with reversed curvatures, as seen in the figures—I am inclined to regard these stouter forms as ripe parasites, ready to escape from the blood-corpuscle.

In one single instance I was so fortunate as to find a doubly-infected blood-corpuscle (fig. 16). The two contained parasites can be seen to be below the normal in size, and one is much smaller than the other; both, however, show abundance of chromatin in the nucleus, and I am inclined to regard their small size as a result of their being stunted in growth owing to lack of sufficient space or nutriment for their proper development.

Free vermicules, as already stated, were scarce in these preparations, and I only saw one which might have been one of the long forms, but as it was obscured by overlying blood-corpuscles, I could not be sure of it. One stumpy form was also found. All the others seen were of what I should term medium length (figs. 22, 23). Their nucleus was similar to the intra-corpuscular forms, but with a tendency to be more compact. But their most marked feature was the presence in their cytoplasm of distinct red-staining granules, apparently of the nature of chromidia; in this point they contrast sharply with the vermicules seen on slides A and B, in which the cytoplasm always appeared free from such granules.

A count of these two slides, carried out in the manner already described, gave the following results:—

	Large intra-corpuscular forms (figs. 19, 20).	Stumpy intra-corpuscular forms (figs. 17, 18).	Vermicules.		
			Stumpy.	Medium (figs. 22, 23).	Long.
Slide C	151	6	0	2	1 ?
Slide D	137	1	1	3	0

These figures, to which must be added the doubly-infected corpuscle shown in fig. 16 from slide C, show that by far the greater proportion of the parasites on these slides are full-grown intra-corpuscular forms.

Comparing the results obtained from all the four slides which I have been able to study, I am inclined to explain the differences between them in the following way:—the parasites on slides C and D (Pl. LVI.) are probably all of the same age, and represent one generation of the parasite, possibly one infection of the host; on the other hand, in slides A and B (Pl. LV.) there are probably two generations of the parasite, resulting, it may be, from two distinct infections of the host, represented respectively by the young forms (figs. 1–5) and the older, free or intra-corpuscular, forms (figs. 6–15). This explanation does not, however, account for the very curious differences between the nuclei seen in the two cases.

No forms of multiplication were seen by me nor by Lt.-Col. Wyville-Thomson, who writes:—"No schizonts were found in either liver or spleen, nor any multiplying forms seen in the blood, all appearing to be at the same stage of development." (These remarks appear, from the sketches made, to apply more particularly to slides C and D.)

Position of the Parasite.—It is evident that the parasite is a typical example of the genus *Hæmogregarina* Danilewsky, in the wide sense; its shortness, relative to the blood-corpuscle which contains it, is one of the points characterising Labbé's genus *Karyolysus*, described originally from lizards, but now known to occur also in other reptiles. It is, however, also characteristic of the species of *Karyolysus* to cause the nucleus of the blood-corpuscle to break up, which this species does not do. In any case the characters by which *Karyolysus* are defined are hardly to be considered of generic value, and its species are best ranked under *Hæmogregarina* until more is known about them. In the species under consideration, I was often struck by its resemblance to the genus *Halteridium* occurring in birds, especially in cases where the parasite is pressed against the nucleus of the blood-corpuscle (compare fig. 7 on Pl. LV.).

Since the species does not appear to have been described before, I propose for it the name *Hæmogregarina thomsoni*, in honour of its discoverer, Lt.-Col. Wyville-Thomson. Should it prove, eventually, to be the case that the parasites on slides A and B (Pl. LV.) are a distinct species from those on slides C and D (Pl. LVI.), I would restrict the name to the latter.

I should like, however, to make a few remarks about the naming of new species of blood-parasites. It is a common practice, which I am here following, to consider a species of blood-parasite as new if it is found in a host in which parasites of that class have not been found or described previously. The assumption underlying this procedure is that different species of animals tend to have distinct species of parasites—an assumption, however, which stands in need of support, for it is quite conceivable that the same species of parasite might infest several species of hosts, and even that the difference of hosts might cause it to appear under forms more or less different in different cases. To regard parasites as being necessarily of distinct species because they occur in distinct hosts is, in reality, not more warranted than the procedure of those naturalists who give distinct names to animals occurring in different geographical regions, even if they do not differ in any other characters, thus begging the question of their specific distinctness. It must be understood, therefore, that the name given to this parasite must be considered as purely provisional and liable to be cancelled, that is merged in some older name, with increased knowledge of these parasites. Names given to parasites distinguished, in the first instance, by the specific distinctness of their hosts must not be taken as necessarily denoting distinct natural species (though this will be true of them in the great majority of cases), but merely, so to speak, as labels affixed to certain classes of objects, whereby they become represented by parts of speech and can be referred to briefly.

So many species of hæmogregarines are now known from various animals that it is almost necessary to apologise for adding another to the list ; I do so with the object of drawing attention to it in the hope that someone may be brought thereby to study its complete life-history. In the present state of our knowledge, it is not new species of hæmogregarines that are needed, but rather new facts about old species.

EXPLANATION OF THE PLATES.

PLATE LV.

Figs. 2-7, 10-12, & 14 are drawn from slide A : all other figures on the Plate are from slide B. All the figures are magnified 2000 linear.

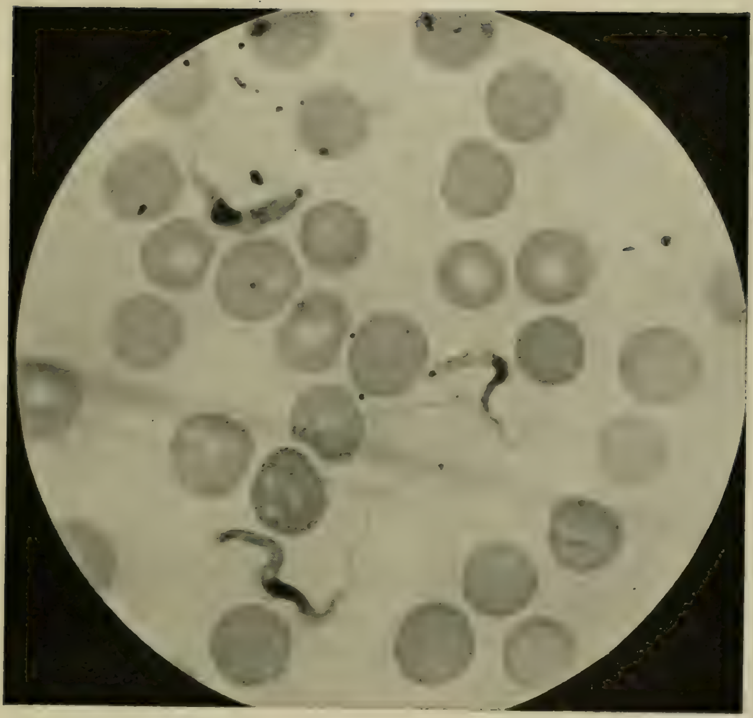
Figs. 1-5, young intra-corpuscular forms.
Figs. 6-8, full-grown intra-corpuscular forms.
Fig. 9, vermicule in the act of leaving the corpuscle.
Figs. 10-15, free vermicules.

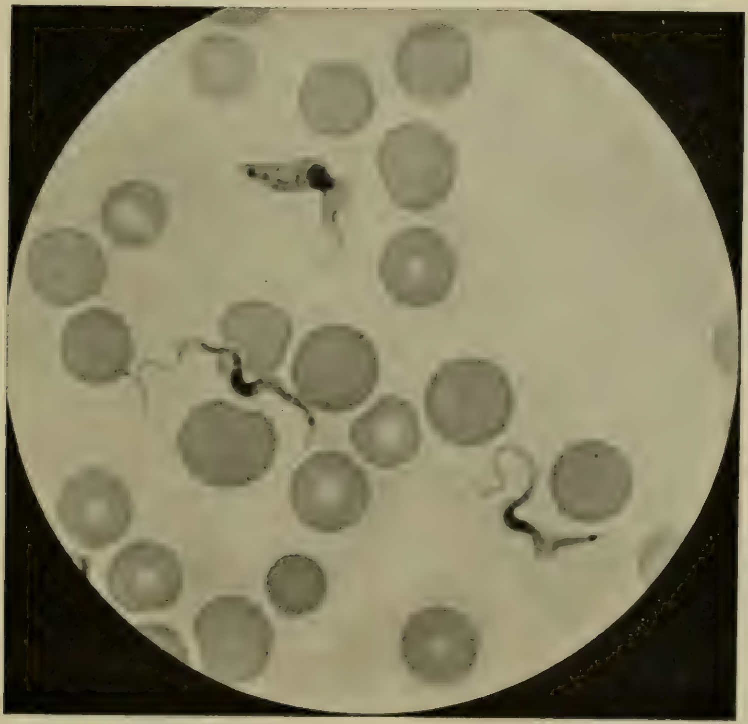
PLATE LVI.

Figs. 16, 17, 19, & 22 are drawn from slide C ; all others from slide D. All the figures are magnified 2000 linear.

Fig. 16, corpuscle infected with two parasites
Figs. 17-21, intra-corpuscular parasites.
Figs. 22, 23, free vermicules.

Lister Institute of Preventive Medicine,
15th Nov., 1907.





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NOTE ON THE POLYMORPHISM OF *TRYPANOSOMA GAMBIENSE.*

BY E. A. MINCHIN, M.A.

Plate XVII.

IN a memoir recently published, Salvin-Moore and Breinl[1] state that when *Trypanosoma gambiense* is examined in the blood "it does not seem possible to detect any true dimorphism or trimorphism." "The three forms often described and alluded to as distinct, consequently appear to be arbitrarily chosen examples in a continuous series of dimensions." It has always seemed to me very remarkable that the great differences in form and structure, no less than in size, between the slender, ordinary, and stumpy forms of *T. gambiense*, differences noted by all competent observers, should have been denied by two authors who claim for their methods of technique a superiority over those employed by all other investigators.

In a preparation of *T. gambiense* from the blood of a rat, which was made by my friend and assistant Dr J. D. Thomson for the cabinet of the Protozoological Laboratory of the Lister Institute, the three typical forms of the trypanosome were found to be very distinctly differentiated. Dr Thomson found and marked two fields in which the three forms occurred in close proximity, so that it was possible to photograph them. The blood-smear was fixed wet with osmic vapour, stained with Giemsa's stain, and mounted in Canada balsam. The two photographs which are reproduced here were taken at a magnification of 1000 diameters by my friend Dr D. J. Reid.

It can be seen clearly from the photographs that the difference between the three forms of *T. gambiense* is by no means one merely of size. The slender form is of great length and has a very long free flagellum. On the other hand the stumpy form is short and its flagellum is also very short, especially the free portion. The ordinary form, which is much the commonest on the slide, is more or less intermediate between the two extreme forms. There is nothing new in these statements, and it seems almost necessary to apologize for restating such obvious facts; it is to be hoped that it will not be necessary to do so again.

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[1] *Annals of Tropical Medicine and Parasitology*, I. (1907), pp. 450, 451; compare also II. (1908), p. 212.

As regards the significance of these forms, I incline to the opinion that the two extremes, slender and stumpy, represent sexual forms differentiated in opposite directions from a neutral type. This view receives support from the observation made by me, that after about 12 hours in the tsetse-fly only slender and stout forms are to be found. Nevertheless the question of the meaning of this well-marked trimorphism cannot be regarded as definitely settled in the present state of our knowledge.

[*Reprinted from the* PROCEEDINGS OF THE ROYAL SOCIETY OF MEDICINE, *March*, 1909.]

The Bacteriology of Summer Diarrhœa.

By H. DE R. MORGAN and J. C. G. LEDINGHAM, M.B.

WE propose in this communication to give a brief review of our knowledge of the bacteriology of summer diarrhœa, dealing more particularly with the results obtained during the last four years by Morgan and other workers in the Lister Institute. Details of bacteriological technique will in great part be dispensed with, as our main object is to elicit discussion on the possibility of correlating current epidemiological opinion on this question with bacteriological findings.

The difficulties experienced by clinicians in accurately differentiating the true summer diarrhœa of children from others occurring during the epidemic season are felt also by the bacteriologist who naturally desires to investigate a series of selected cases of the disease concerning which clinical opinion is unanimous. Unfortunately, this desideratum can never be completely realized. The cases vary greatly in severity in the same season and in different seasons, and accordingly the bacteriologist has to control the results obtained in a selected group of clinically typical cases with those obtained in extensive control series of less severe diarrhœas, diarrhœas occurring as complications of other diseases, and, finally, in a series of apparently healthy children.

The first attempt on a large scale to investigate the bacteriology of summer diarrhœa was made as long ago as 1887 by Booker in America. Up to that time the flora of the intestinal tract had received but scanty attention. Only two years before, in 1885, the *Bacillus coli communis* and the *Bacillus lactis aerogenes* had been described by Escherich and for some time the importance of these micro-organisms was considered to be a dominant one.

The work done by Booker was very complete so far as bacteriological methods permitted, but with our present knowledge little or no etiological significance can be attached to the micro-organisms isolated by him in summer diarrhœa. Booker himself felt that no one micro-organism of the many varieties recovered from his cases could be considered as the specific agent. In an attempt to correlate his bacteriological findings with clinical phenomena he adopted the following grouping:—

Group I, 35 cases: The patients in this group are emaciated and exhibit toxic phenomena. The stools are at times frequent and at other times infrequent. Among other bacilli isolated (including the *Bacillus coli communis*) the *Bacillus proteus vulgaris* appears to occupy an outstanding position.

Group II, 27 cases: The cases are serious and show general toxic disturbance. The stools are fluid or soft, often green and slimy, and vary in frequency from three to twenty in twenty-four hours. Micrococci (streptococci) preponderate in these stools, in conjunction with pus-cells.

Group III, 6 cases: The cases are serious, the stools vary in frequency and often have a putrid odour. The *Bacillus coli communis* is numerous in all these cases along with the *Bacillus lactis aerogenes*. A large number of inconstant varieties are also found.

Group IV, 24 cases: The cases are mild and of short duration. The *Bacillus coli* predominates in all cases and occurs sometimes in pure culture.

Some general observations made by Booker with regard to the distribution of coliform organisms in the intestinal tract and in the fæces are of interest. He noted that while the *Bacillus coli communis* and the *Bacillus lactis aerogenes* do not altogether disappear from diarrhœal fæces, they occur in much fewer numbers than in the fæces of healthy milk-fed children, and, in fact, tend to diminish in proportion to the severity of the case, their place being taken by varieties which occur inconstantly in the healthy intestine. This substitution of the normal flora by inconstant varieties in diarrhœal fæces will be referred to later.

In considering the work of American authors on the diarrhœas of children one must pay close attention to the nature of the cases they dealt with. It will be mentioned below that the clinical picture of summer diarrhœa in many of the epidemics investigated in America is entirely different from that which prevails in this country. One very crucial point of distinction is the condition of the stools. In cases met with in London the stools may be characterized generally as green, watery, slimy and putrid with varying acid or alkaline reaction. Blood and mucus are rarely, if ever, found. Unlike most of the cases subsequently investigated in America, Booker's cases did not present blood or mucus in the stools, and they are, accordingly, clinically comparable with our summer cases in London. As, however, the dysentery bacillus of Shiga was not described until 1898, it is impossible now to know whether Booker's cases may have been associated with bacilli of the dysentery group.

The next advance made in the bacteriology of infantile diarrhœa was made in 1903 in America, when the subject was investigated by a number of observers, notably Wollstein, Park, Collins and Goodwin, Flexner, Duval and Schorer, Weaver and Tunnicliffe. The Flexner type of the dysentery bacillus was discovered in 1899, and was at this time not completely differentiated from that of Shiga described in 1898. The object of the investigations above referred to was to determine whether these infantile diarrhœas were also associated with dysentery organisms. The clinical features in most of the epidemics recorded were very similar. The stools invariably contained mucus, and as a rule an admixture of blood.

Wollstein examined the stools of 114 cases of infantile diarrhœa, and isolated the dysentery bacillus (Flexner type) in 39 cases. From all those cases which showed both blood and mucus in the stools, the dysentery bacillus was recovered. Agglutination reactions with the sera of the patients were obtained in a large percentage of the positive cases, but not during the first week of the disease. In autopsies the dysentery bacillus was never found outside the intestinal tract.

In the same year (1903) Park Collins and Goodwin examined cases of summer diarrhœa associated with excessive mucus and occasional blood. The majority of such dysenteric cases showed Flexner bacilli in the stools. Duval and Schorer (1903) reported the finding of dysentery bacilli in 94 per cent. of summer diarrhœa cases, including all grades of severity. They did not believe that the character of the stools was a certain indication of the presence of dysentery bacilli, but they admitted that these bacilli were most likely to be present in cases accompanied by bloody mucus. In opposition to Wollstein they also isolated dysentery bacilli from the stools of normal infants, but gave no figures in support of their statement.

Although normal children living amongst infected cases might quite conceivably harbour dysentery bacilli, we must also reckon with the possibility that these dysentery bacilli from normal infants may not have been true dysentery bacilli, but paradysentery bacilli similar to those occasionally got by Morgan, and to be later referred to. Other American epidemics associated with dysentery bacilli were reported by Cordes, Weaver, Tunnicliffe, and others. It may be noted that Cordes got a positive agglutination reaction (1 in 40 to 1 in 50) with the sera of 22 per cent. of his cases.

That the dysenteric cases of summer diarrhœa occurring in America are due to infection by bacilli of the dysentery group is thoroughly established. Only scanty attention, however, has been paid to the bacteriology of the non-dysenteric cases (so-called cholera infantum) similar to those occurring in this country. Park, Collins and Goodwin examined some cases of cholera infantum, but failed to isolate dysentery bacilli. They also failed to recover the dysentery bacillus from cases of subacute diarrhœa in institutions where no cases of the dysentery type existed. Also, the blood-serum of cases of cholera infantum has no agglutinating effect on dysentery bacilli in dilutions higher than 1 in 10.

Schwarz confined his attention to cases of typical summer complaint which were characterized by profuse watery, green stools with little or no mucus. Dysentery bacilli were not recovered from any one of the thirty such cases examined, and the serum reactions were all negative.

In 1905 Jehle and Charleton, in Vienna, examined numerous cases of acute gastro-enteritis (cholera infantum) during the hot weather, but never found dysentery bacilli. Agglutination tests also proved negative.

In this survey we have thought fit to mention only those bacteriological investigations on summer diarrhœa which deserve attention, from the fact that they have been made on an extensive scale and scientifically worked out. Isolated observations on single cases or small groups of cases are of little value, and will not be referred to.

Summer Diarrhœa in London.

We shall now proceed to an analysis of the results obtained by Morgan and others in the bacteriological investigation of summer diarrhœa in London during the four years 1905-1908. In these researches chief attention has been paid to that group of the intestinal bacteria which does not ferment lactose. To this group all the pathogenic bacteria associated with affections of the intestinal tract belong—viz., the typhoid and paratyphoid bacilli, the organisms of the food-poisoning group and the dysentery group.

Reports of Morgan's work during the epidemic seasons of 1905 and 1906 have already been published, but it will be convenient to discuss the results of these two years with those of 1907 and 1908, when researches on an extensive scale were carried out, demanding the labour of many laboratory workers and the kindly co-operation of hospital physicians and health authorities.

SOURCE AND AMOUNT OF MATERIAL EXAMINED DURING THE FOUR YEARS.

1905: The fæces of 58 cases of infantile diarrhœa under treatment at the Hospital for Sick Children, Great Ormond Street, and the Victoria Hospital, Chelsea, were examined bacteriologically, along with any available post-mortem material. Of these 58 cases the clinical diagnosis in 28 was acute infective diarrhœa, and in 30 catarrhal diarrhœa, but the stools in the latter cases did not differ appreciably in appearance from those of the acute cases.

1906: During the epidemic season the fæces and autopsy material of 54 cases from the special ward set apart for diarrhœa at the Hospital for Sick Children, Great Ormond Street, were investigated.

1907: Preparations were made this year for a very extensive investigation of the subject, but, unfortunately for the purposes of research, very few cases occurred, and these, as the clinicians agreed, were atypical and of only moderate severity. The material came from Paddington Green, Shadwell, Great Ormond Street, and Victoria Hospitals; 191 cases in all were examined, both in-patients and out-patients. Material from ten autopsies came also under review. Many of the cases were examined repeatedly.

1908: One hundred and sixty-six selected cases of summer diarrhœa occurring at Shadwell and Victoria Hospitals were investigated. When possible the stools of each patient were taken for examination on three consecutive days, and in the event of the patient's death complete bacteriological examination of the organs was made. Material from thirty-three autopsies was available.

BRIEF DESCRIPTION OF TECHNIQUE.

Whatever the nature of the material, the technique followed remained essentially the same. In the case of fæces, intestinal scrapings at autopsies, portions of various organs, &c., an emulsion of the material was made in sterile broth. A loopful of the emulsion was then spread over three plates containing MacConkey's bile-salt-lactose neutral red agar. The colourless (or non-lactose) colonies which developed were picked off and submitted to a series of biological and chemical tests—viz., indol-formation, liquefaction of gelatin, motility and fermentation reactions on various sugar media. In 1908 mannite-bile-salt agar was employed for reasons stated below (*see* Tables I and II), pp. 148 and 149.

The annexed table (Table I) shows all the varieties of non-lactose, non-liquefying micro-organisms met with in the fæces of children suffering from summer diarrhœa, and the percentages in which they occur. The biological characters of these micro-organisms have been completely worked out, and they have been classified according to their affinities to well-known pathogenic members of this group. During the years 1905, 1906, 1907, the exact percentages of these micro-organisms were ascertained, but during 1908, owing to the employment of a modified medium (mannite-bile-salt agar), which was justified by previous experience of the predominance of Morgan's bacillus, only the non-mannite group, to which this bacillus belongs, came under review. We do not propose to discuss in detail all these various micro-organisms, many of which may be familiar to those experienced in the bacteriological examination of excreta. Some special points, however, deserve attention.

In normal fæces the non-lactose group lags in frequency far behind the lactose-fermenting group, of which the *Bacillus coli communis* may be taken as a type. During the investigation of fæces from series of normal persons, adult and infantile, one finds colourless or non-lactose colonies only in a small proportion of the cases. The predominant group, at least in normal stools, is that of the lactose fermenters.

In diarrhœal cases, on the other hand, the lactose-fermenting group is still met with, but in less marked frequency; while the non-lactose varieties relatively increase, and may even in rare cases lead to the complete exclusion of the coliform group.

It will be seen, from the columns giving the percentages of the various non-lactose fermenters met with in the four years' work, that the bacillus designated No. 1, which will be hereafter referred to as Morgan's bacillus, predominates conspicuously over all the other varieties. The group to which it belongs—*i.e.*, the non-mannite, non-liquefying group—is an exceedingly small one. The only known pathogenic member of this group is the dysentery bacillus of Shiga, which, of course, is readily distinguishable from Morgan's bacillus by at least three important characteristics—viz., absence of motility and absence of indol-formation and gas-production.

The second group possesses some affinities to the Flexner type of the dysentery bacillus and to the *Bacillus typhosus*—at any rate in so far as the fermentation reactions on glucose and mannite are concerned—but their reactions on litmus-milk, and their properties with regard to indol-formation, serve to differentiate them readily.

(Table II shows the reactions of the well-known pathogenic members of the non-lactose group.)

The Bacillus No. 3, unlike Flexner's, has no action on litmus-milk, and it produces acid in sorbite, which the Flexner bacillus never does. From the small percentages in which these paradysentery bacilli occur, there is no evidence that dysentery or pseudodysentery bacilli have any significance in the etiology of summer diarrhœa, as it is met with in London.

The Gaertner group, of which there are several varieties (judging by fermentation reactions alone), is headed by the true *Bacillus enteritidis* of Gaertner (isolated once in 1905, four times in 1906, and three times in 1907).

The remaining groups require little attention. Groups X, XII, XIV, and XV are common frequenters of normal and adult stools, and Group X is often isolated from drinking water. Group XV, a variety which does not possess any action on sugars, corresponds to the *Bacillus fæcalis alkaligenes* of Petruschky.

OBSERVATIONS ON THE EPIDEMIC SEASONS.

It will be apparent that Morgan's bacillus occupies a predominant place among the non-lactose fermenters in epidemic diarrhœa. The fact that this bacillus was so conspicuous in the years 1905-6 led to the extensive investigations of 1907-8. So far as mere association of the bacillus with the disease is concerned, this point was proved absolutely, as the figures show. The epidemic of 1907 deserves special mention. The research in that year extended from the first week of August to the first week of October. During the first three weeks only six cases were examined, and Morgan's bacillus was not isolated. It was not till the ninth week of the research (last week of September) that Morgan's bacillus began to be isolated with frequency from the cases examined. The percentage of success in that week reached 56·5. An interesting point in this connexion is the fact that the mortality curve for summer diarrhœa in London (1907) gradually rose to a maximum during the last week of September, whereas for the preceding five years the average mortality curve showed a maximum rise in August. Clinicians were also agreed that this last week of September was the only true epidemic period in the summer of 1907.

EXAMINATION OF AUTOPSY MATERIAL.

In 1906 Morgan's bacillus was isolated from the intestinal tract in three cases, and once from the mesenteric glands. It was never isolated from the spleen in 1906. (N.B.—Invasion of the spleen occurred in feeding experiments on young rats and young rabbits, but not in monkeys.) In 1907 material was obtained from ten autopsies. In two only of these cases had Morgan's bacillus been found in the fæces *intra vitam*, and it was in one of these two that Morgan's bacillus was recovered from the spleen. The others were negative. In 1908 Morgan's bacillus was recovered in sixteen autopsies out of thirty-three. It may be convenient to detail the sites in which the bacillus was found: jejunum once, colon thirteen times, liver twice, spleen, kidney and mesenteric glands three times, lung once, bile twice, urine three times, and heart blood three times. In some cases, therefore, it would appear that Morgan's bacillus may give rise to a septicæmia.

EXAMINATION OF NORMAL FÆCES OF ADULTS AND CHILDREN.

Throughout all four seasons the possibility of the presence of Morgan's bacillus in the fæces of other than diarrhœal cases has been borne in mind, and numerous control series have been investigated. The results in these cases may be briefly summarized. In 1905, during the winter, 20 normal children were examined, and Morgan's bacillus was found in one case. In 1908, during the epidemic season, 99 normal children were examined and the bacillus was isolated twelve times (12 per cent.). Of these cases, 60 were examined by Dr. Eyre and Dr. Minett at Guy's Hospital during the season of 1908, and four positive cases were found (= 6·6 per cent). The remaining 39 cases came from the Paddington district, and of these eight harboured Morgan's bacillus (= 20 per cent.). Adults: 90 cases were examined during the winter of 1907-8, and the bacillus was obtained in one case only; 33 cases were examined in the spring of 1908, and the bacillus was recovered once.

Children dying from other Diseases.

During the winter 1907-8 post-mortem material from 30 cases was examined, and Morgan's bacillus was recovered in four. The cause of death was broncho-pneumonia, and two of these four had had diarrhœa.

Children suffering from other Diseases, with Diarrhœa as a Complication.

In the season of 1908 22 such cases were examined, and the bacillus was recovered in two cases. Morgan's bacillus is therefore met with in a small percentage of cases of healthy children and of children suffering from other diseases complicated by diarrhœa. Adults only very rarely harbour the bacillus. It is to be noted, also, that the percentage of carrier cases is less during the winter season than during the epidemic season, when, it may be presumed, facility of contact is much greater.

Examination of Milk, Flies and other material which may act as Vehicles of Infection.

Milk.—In view of the fact that milk has been considered by many observers to be the vehicle for the transference of the infection of summer diarrhœa to children, attempts have frequently been made on an extensive scale to isolate Morgan's bacillus from milk samples. In the beginning of the season 1907 samples of milk from feeding-bottles, swabs from rubber teats and comforters were examined, but negative results were invariably obtained. During the season 1908 samples of milk from twenty-nine infected houses in Paddington and from twenty-six uninfected houses in the same district were examined, but only in one instance (from an infected house) was Morgan's bacillus isolated. Such a result was puzzling, especially when one considered the prevailing view of the transference of infection by milk. Some laboratory experiments made in this connexion throw a little light on this matter. When unsterilized milk was inoculated with Morgan's bacillus and incubated at room temperature for six, twelve, twenty-four hours, it was impossible to recover the bacillus by the usual methods. In sterilized milk the bacillus grew readily and was easily recovered. Similar experiments with condensed milks have not so far been made.

Flies.—A good deal of attention has been paid within the last two years to the conveyance of infection by the common house-fly. The matter was considered in detail by the London County Council in 1907 and reported on by Dr. Hamer in 1908. He came to the conclusion that flies could not be the sole agents for the conveyance of summer diarrhœa from patient to patient because flies were found in houses in the late autumn after the epidemic had ceased. It must be remembered, however, that, with the onset of cold weather, flies become much more sluggish in their movements and would therefore be less likely to spread infection rapidly. Batches of flies came for examination from infected and uninfected houses in Paddington and from a country house situated many miles from London, where no cases of diarrhœa had occurred, at any rate within a radius of two miles. The flies were killed with ether vapour and crushed with a sterile rod in peptone broth. The result was that Morgan's bacillus was isolated from nine of the thirty-six batches from infected houses and from one of the thirty-two batches from uninfected houses. It was also got in five out of twenty-four batches from the country house.

Dust.—Samples of dust (from ashbins) collected from eleven infected houses in Paddington were examined, with negative results.

Cow and Horse Fæces.—Morgan's bacillus was isolated once from fresh cow's fæces (eighteen animals examined). It has not been recovered from the fæces of the horse (thirteen animals examined).

PATHOGENICITY OF MORGAN'S BACILLUS FOR LABORATORY ANIMALS.

In 1905 feeding experiments were carried out with young rats and young rabbits. Death took place within twenty-four hours and was preceded by violent diarrhœa. The bacillus was recovered from the spleen and sometimes from the heart blood.

In 1906 Morgan's bacillus was again virulent for rats by feeding. Four small but full-grown monkeys received with their food one agar tube each of Morgan's bacillus. All four died after a period of diarrhœa which came on from two to twelve days after feeding. The diarrhœa was an acute and progressive one and was followed by rapid emaciation and death. No vomiting was observed.

The strains of Morgan's bacillus isolated during 1907 and 1908 were, for some reason still unexplained, much less virulent for experimental animals by feeding than those recovered in previous years. The monkeys which were fed with Morgan's bacillus in 1908 developed diarrhœa which lasted seven to eight days, but all ultimately recovered.

TOXIN PRODUCTION.

This subject will be only very briefly referred to. Filtrates of broth cultures of Morgan's bacillus grown for one month are toxic for the guinea-pig, the minimum lethal dose by intraperitoneal injection being 5 c.c. The toxin can be concentrated by precipitation, so that the minimum lethal dose becomes 0·5 c.c. to 1 c.c. Great difficulties have been experienced in the immunization of the horse and donkey with Morgan's bacillus, but a serum has been obtained which possesses marked antitoxic properties.

AGGLUTINATION REACTIONS WITH THE SERA OF PATIENTS.

During the first season, 1905, forty-four samples of serum were tested against known micro-organisms like the *Bacillus typhosus* and the *Bacillus enteritidis* of Gaertner. In two cases only was a reaction obtained (1 in 60) with these micro-organisms. Also one case in which Morgan's bacillus was present gave a reaction (1 in 30) with the *Bacillus typhosus*.

In 1908 this question was attacked in a more extensive fashion. Samples of serum were taken at various stages of the disease and during convalescence. Each specimen was tested against various strains of Morgan's bacillus, including the homologous strain when possible, and also against two or three strains of the *Bacillus proteus vulgaris*. Sixty-five patients in all were examined, and thirty of these gave a positive reaction with Morgan's bacillus. One gave a reaction with the *Bacillus proteus vulgaris*. Of twelve healthy control children only one gave a slight reaction (1 in 10) with Morgan's bacillus. The dilution in which a reaction was obtained never exceeded 1 in 40, and as a rule 1 in 20 was the limit. The best results were obtained at or near convalescence. Of nineteen patients at this stage fifteen gave a positive reaction (78·9 per cent.). Of twenty-seven sera taken within a few days of admission only five gave a positive reaction. At this early stage agglutinin-development is, as one might expect, slight. In a few instances where repeated samples of blood were taken during the progress of the disease, it was noticed that agglutination occurred in gradually higher dilutions as convalescence approached, and in gradually decreasing dilutions after the child had left hospital till a reaction was no longer obtained. The longest period after convalescence at which a reaction appeared was thirty days, and in one case it had disappeared thirty-seven days after convalescence. Of the sixty-five patients examined, Morgan's bacillus was recovered in forty-two, and of these forty-two cases a reaction was obtained in twenty-four. Of the remaining twenty-three cases from which Morgan's bacillus was not isolated six only gave a reaction. It should be noted that those cases which gave no reaction with the homologous strains frequently agglutinated stock strains of the bacillus, and vice versa.

THE QUESTION OF A FILTER-PASSER AS THE INFECTIVE AGENT.

During the season 1908 some preliminary experiments were performed with the view of testing this hypothesis. The blood and organs from three typical fatal cases of summer diarrhœa were mashed up in salt solution and passed through a Berkefeld filter. With this material feeding experiments in young cats and inoculation experiments in rats were made. The results were invariably negative.

GENERAL SUMMARY AND CONCLUSIONS.

We have, in the above statement, attempted to give a brief sketch of the very laborious researches which Morgan and other workers have carried out during the last four years on the bacteriology of summer diarrhœa. The necessity for making a careful research in the non-lactose group for some specific micro-organism in this disease was, as we have already indicated, justified by the knowledge which has accumulated concerning typhoid, paratyphoid, dysentery and food-poisoning bacilli. The outcome of this search has been that a certain bacillus (Morgan's bacillus) has been proved to occupy a predominant position among the non-lactose fermenters in the excreta of summer diarrhœa patients. In selected cases of the disease it has been isolated in the very high percentage of 63—a percentage which compares very favourably with that obtained—*e.g.*, in typhoid. Further, in the course of this work a large mass of information has been obtained concerning a very important group of the intestinal flora, and this, it is hoped, will prove of great value to future workers on these lines. To one searching for well-known members of the pathogenic non-lactose group in the excreta, the convenience of having at one's disposal such a table as that shown you for constant reference is obvious. The association of Morgan's bacillus with summer diarrhœa has, we think, been abundantly established. Mere association, however, does not necessarily prove etiological connexion, though it must form a very important link in any chain of evidence connecting a bacillus with an infection. The fact that this bacillus is found in a small percentage of healthy children both during and apart from the epidemic season, and in a still smaller percentage of adults, does not conflict with present-day notions regarding the natural history of pathogenic micro-organisms affecting the intestinal tract. That acute diarrhœa of the summer type may also occur sporadically during the winter season is well known, and it seems quite reasonable to attribute such cases to contact infection from carriers. Extended observations on the duration of the bacillus in the intestine of convalescents are at present being made, and they are expected to yield valuable results.

With regard to the pathogenicity of Morgan's bacillus for experimental animals, we have found that rats and monkeys are susceptible to infection by feeding and, after a period of diarrhœa, succumb. Some strains of the bacillus appear to be much less virulent than others in this respect. Whether the virulence of the strain can be correlated with the severity of the epidemic, we are not yet in a position to decide. By experimental inoculation, both the bacillus and its toxin are markedly pathogenic for rats, guinea-pigs and goats.

It is, however, on the score of association, pathogenicity by feeding and otherwise (especially the results with monkeys), and the serum-reactions on the part of the infected hosts, that the claim of Morgan's bacillus to be considered an important etiological factor in summer diarrhœa mainly rests. Whether that claim will ultimately find general acceptance it is not for us to predict. We are fully alive to the many problems which its habitats outside the infected host suggest. At any rate, this bacillus is in no danger of dying out for want of an animal host to carry it on from one season to another.

The presence of the bacillus in flies from infected houses has an importance which is not necessarily lessened by the fact that flies, in uninfected houses of the same district, may also harbour the bacilllus. The intercourse that may occur between flies belonging to the same district is a question on which one cannot dogmatize.

We are at present unable to offer any explanation of the positive results obtained in flies from the country, though there is some evidence that Morgan's bacillus may be found in the bovine intestine. If this proves to be the case, the question of milk infection will assume another phase.

From the actual bacteriological results obtained, we are inclined to the view that during the epidemic season direct infection by contact with carriers or infected flies may play as important a role as infection through the medium of the milk. Sporadic cases in the winter can only be explained on a carrier hypothesis.

In conclusion, we desire to thank all those physicians, medical officers of health, nurses and sanitary inspectors whose generous co-operation and interest made these researches possible.

Our special thanks are due to Dr. Dudfield, Medical Officer of Health of Paddington, for the very valuable assistance he has given us in the collection of specimens.

It may be mentioned that a detailed report of the bacteriological work in connexion with the investigation of summer diarrhœa will appear later in the *Journal of Hygiene.*

Dr. MORGAN: I have practically nothing to add to Dr. Ledingham's and my joint communication, for although it is only a sketch of the results of four years' work I think it covers nearly all the ground. As regards the association of Morgan's bacillus with the disease, we have found that it is isolated much more frequently from severe cases of summer diarrhœa than it is from mild ones. This was shown by the low percentage in which it was isolated in 1907, when the epidemic was of a mild type. It is still more forcibly shown by the figures obtained during 1908: Seventy-four severe cases were examined from Shadwell Hospital with a mortality of 45·9 per cent., and from these Morgan's bacillus was isolated in 63·5 per cent., whereas from the fifty mild cases from Paddington, of whom none died, this bacillus was only isolated in 34 per cent. Of course, there still remains a great deal of work to be done in relation to this bacillus; at present we are investigating the "carrier" question—in other words, we are examining the stools of the children from whom we isolated Morgan's bacillus last summer and who recovered, to see whether the bacillus is still present; we are also trying to find the best method of producing an efficient antitoxin. The main object, however—that of stamping out the disease—can only be effected by the co-operation of the various sanitary authorities, and it is to them we look for help, not without confidence.

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Table I.—Bacilli that neither Ferment Lactose nor Liquefy Gelatine.

Bacilli		Morphology	Glucose	Mannite	Dulcite	Lactose	Cane sugar	Litmus-milk			Indol	Sorbite	Gelatine	Percentage in			
								First day	Third day	Fifteenth day				1905	1906	1907	1908
Type I	1	M.B.	A.G.	–	–	–	–	O.	O.	Alk.S.	+	–	–	48·2	55·8	16·2	53·0
	1A	N.M.B.	A.G.	–	–	–	–	O.	O.	Alk.S.	+	–	–	0·0	0·0	0·5	
	2	N.M.B.	A.G.	–	–	–	–	O.	O.	Alk.S.	–	–	–	5·6	0·0	4·7	
Type Flexner	3	N.M.B.	A.	A.	–	–	–	O.	O.	O.	+	A.	–	8·6	8·8	5·2	
	4	N.M.B.	A.	A.	–	–	–	O.	O.	O.	–	–	–	3·4	2·9	1·05	
	4A	N.M.B.	A.	A.	–	–	–	A.	A.	A.C.	–	–	–	0·0	14·7	6·8	
	4B	M.B.	A.	A.	–	–	–	A.	O.	Alk.	+	–	–	0·0	2·9	0·0	
	4C	M.B.	A.	A.	–	–	–	A.	O.	Alk.	–	–	–	0·0	2·9	0·0	
Type V	5	M.B.	A.	–	–	–	–	O.	O.	Alk.S.	+	–	–	6·9	11·7	8·3	
	5A	N.M.B.	A.	–	–	–	–	O.	O.	Alk.S.	+	–	–	0·0	0·0	3·6	
	5B	M.B.	A.	–	–	–	–	A.	Alk.S.	Alk.	+	–	–	0·0	0·0	0·5	
Type Gaertner	6	M.B.	A.G.	A.G.	A.G.	–	–	A.	O.	Alk.	–	A.G.	–	1·7	11·7	1·6	
	7	M.B.	A.G.	A.G.	A.G.	–	–	A.	Alk.	Alk.	+		–	3·4	11·7	1·6	
	7A	N.M.B.	A.G.	A.G.	A.G.	–	–	A.	O.	A.C.	+		–	0·0	2·9	2·6	
	7B	N.M.B.	A.G.	A.G.	A.G.	–	–	A.	O.	Alk.	+		–	0·0	0·0	5·7	
	7C	M.B.	A.G.	A.G.	A.G.	–	–	O.	A.C.	A.C.	+		–	0·0	0·0	3·6	
	8	N.M.B.	A.G.	A.G.	A.G.	–	–	A.	O.	A.	–		–	1·7	0·0	0·0	
	9	N.M.B.	A.G.	A.G.	A.G.	–	–	A.	A.	Alk.	–		–	1·7	0·0	0·0	
Type X	10	N.M.B.	A.G.	A.G.	A.G.	–	A.G.	A.	A.	A.C.	+		–	6·9	2·9	0·0	
	11	M.B.	A.G.	A.G.	A.G.	–	A.G.	O.	O.	Alk.	+		–	5·1	5·8	3·6	
	11A	M.B.	A.G.	A.G.	A.G.	–	A.G.	A.	A.	Alk.	–		–	0·0	8·8	0·0	
	11B	M.B.	A.G.	A.G.	A.G.	–	A.G.	O.	A.	A.C.	+		–	0·0	2·9	0·0	
	11C	N.M.B.	A.G.	A.G.	A.G.	–	A.G.	O.	O.	Alk.	+		–	0·0	0·0	4·2	
Type XII	12	M.B.	A.G.	A.G.	–	–	A.G.	A.	A.	A.C.	–		–	3·4	0·0	0·0	
	13	M.B.	A.G.	A.G.	–	–	A.G.	A.	A.	A.C.	+		–	1·7	8·8	0·0	
	13A	N.M.B.	A.G.	A.G.	–	–	A.G.	A.	A.	A.C.	+		–	0·0	5·8	0·0	

Type XIV	14	M.B.	A.G.	A.G.	–	–	–	A.	A.	Alk.S.	+		–	8·6	2·9	1·05
	14A	N.M.B.	A.G.	A.G.	–	–	–	A.	Alk.	Alk.	+		–	0·0	8·8	3·2
	14B	M.B.	A.G.	A.G.	–	–	–	A.	A.	A.C.	+		–	0·0	8·8	2·1
	14C	N.M.B.	A.G.	A.G.	–	–	–	A.	O.	A.C.	+		–	0·0	5·8	1·05
	14D	M.B.	A.G.	A.G.	–	–	–	A.	A.	Alk.	–		–	0·0	0·C	0·5
Type XV	15	N.M.B.	–	–	–	–	–	Alk.S.	Alk.S.	Alk.	–		–	?	?	0·5
	15A	M.B.	–	–	–	–	–	Alk.	Alk.	Alk.	–		–	?	?	1·6
	15B	N.M.B.	–	–	–	–	–	O.	O.	O.	–		–	?	?	2·1

TABLE II.—SOME KNOWN PATHOGENIC INTESTINAL BACTERIA.

Bacillus	Morphology	Glucose	Mannite	Dulcite	Lactose	Cane sugar	Litmus-milk			Indol	Sorbite	Gelatine
							First day	Third day	Fifteenth day			
Bacillus dysenteriæ (Flexner, Philippines)	N.M.B.	A.	A.	–	–	–	A.	Alk.S.	Alk.S.	+	–	–
Bacillus dysenteriæ (Shiga) ...	N.M.B.	A.	–	–	–	–	A.	Alk.S.	Alk.S.	–	–	–
Bacillus typhosus	M B.	A.	A.	–	–	–	A.	A.	A.	–	A.	–
Bacillus enteritidis (Gaertner) ...	M.B.	A.G.	A.G.	A G.	–	–	A.	Alk.	Alk.	–	A.G.	–
Bacillus paratyphosus A (Schotmüller)	M.B.	A.G.	A.G.	A.G.	–	–	A.	A.	A.	–	A.G.	–
Bacillus paratyphosus B (Schotmüller)	M.B.	A.G.	A.G.	A.G.	–	–	A.	Alk.	Alk.	–	A.G.	–
Bacillus hog cholera (McFadyean) ...	M.B.	A G.	–	–	–	–	A.	A.	A.	–	–	–

M B. = Motile bacillus.   N.M.B. = Non-motile bacillus.   A. = Acid.   Alk. = Alkaline.   S. = Slight.   G. = Gas.

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Further Results of the Experimental Treatment of Trypanosomiasis: being a Progress Report to a Committee of the Royal Society.

By H. G. PLIMMER, F.L.S., and H. R. BATEMAN, Captain R.A.M.C.

(Communicated by J. Rose Bradford, M.D., F.R.S. Received August 25, 1908.)

The following results are a continuation of the work of which summaries have already appeared in the 'Proceedings of the Royal Society.'*

The experiments have been carried out with the same strains of Nagana and Surra as were used before.

A.—*Condition of the Animals living at the Date of the Completion of the Tables in the last Paper.*

Table I.—Nagana Rats treated with Atoxyl and Succinimide of Mercury.

(Average duration of untreated disease, 5·5 days.)

No. 4	died on the	307th	day after inoculation.
„ 7	„	365th	„
„ 10	„	249th	„
„ 15	„	188th	„
„ 21	„	63rd	„

Of these, No. 15, which was apparently cured, was used on the 147th day after inoculation for re-inoculation, with the view of ascertaining if any immunity had been conferred. This was found not to be the case.†

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* B, vol. 79, 1907, pp. 505—516, and B, vol. 80, 1908, pp. 1—12.

† *Vide* 'Roy. Soc. Proc.,' B, vol. 80, p. 10.

None of the above died with any of the signs of Nagana. Of the 21 rats tabulated,* only one died from trypanosomiasis, and this one was probably atoxyl-proof.

Table III.—Surra Rats treated with Atoxyl and Mercury Sozoiodol.

No. 5 died on the 286th day after inoculation from broncho-pneumonia.

Table VI.—Surra Rats treated with Atoxyl and Iodopin.

No. 9 died on the 221st day after inoculation from worms.

Rats treated with Atoxyl and Liq. Hydrarg. Perchlor.

The rat still living at the date of the last paper died on the 187th day after inoculation from broncho-pneumonia.

Rats treated with Sodium Antimonyl Tartrate. (Table on pp. 8—9 of the last Paper.)† *Results to August* 20.

No. 7 *is living and well* 354 *days after inoculation.*
„ 8 died on the 194th day „
„ 9 „ 323rd „ „
„ 10 „ 145th „ „
„ 14 „ 48th „ „
„ 15 „ 67th „ „
„ 16 „ 227th „ „
„ 17 „ 227th „ „
„ 19 „ 161st „ „
„ 20 „ 49th „ „
„ 21 „ 210th „ „
„ 23 „ 104th „ „
„ 25 „ 134th „ „
„ 26 „ 134th „ „
„ 27 „ 33rd „ „
„ 28 „ 34th „ „
„ 29 „ 57th „ „
„ 30 „ 28th „ „
„ 31 „ 147th „ „
„ 32 *is living and well* 346 *days after inoculation.*
„ 33 died on the 228th day „
„ 34 „ 188th „ „
„ 35 *is living and well* 346 *days after inoculation.*
„ 36 died on the 228th day after inoculation.
„ 38 „ 173rd „ „

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* 'Roy. Soc. Proc.,' B, vol. 79, p. 510.

† 'Roy. Soc. Proc.,' B, vol. 80

It will be noticed that nine of the above rats lived for over 200 days, and nine others considerably over 100 days. Of those which have died only four have had recurrences (two had one, and two had three recurrences); none of them died with any symptoms of trypanosomiasis, and in none were trypanosomes found after death. An emulsion of the liver and of the bone-marrow of Nos. 9, 16, 17, 21, and 25 was injected into other rats with negative results in every case.

Rats treated with equal parts of 1 *per cent. Solutions of Sodium Antimonyl Tartrate and Sodium Arsenyl Tartrate.*

The five rats living at the date of the last paper have died :—

No. 1 died on the 199th day after inoculation.
„ 2 „ 26th „
„ 3 „ 43rd „
„ 4 „ 59th „
„ 5 „ 40th „

As was anticipated from the earlier experiments, this treatment has no advantages over that with antimony alone.

B.—*Further Experiments.*

Potassium Antimonate.

This was tried in doses up to 7 minims of a 1 per cent. solution. In this dose it was poisonous to rats, and it did not kill the trypanosomes.

Atoxyl and Sodium Antimonyl Tartrate.

This was given in doses of 5—7 minims of a 5 per cent. solution of atoxyl and of a 1 per cent. solution of sodium antimonyl tartrate. Three Nagana rats lived respectively 23, 43, and 41 days; they all had recurrences (the last had five) and died with living trypanosomes in the blood.

Pushing the drug does not have any good effect (see next table); the trypanosomes are not driven out more quickly, or more effectually; recurrences are more common, and inflammatory intestinal lesions were present in nearly every case. Two rats died with living trypanosomes in the blood: these two had become antimony-proof, as the later doses did not remove the trypanosomes from the blood, nor make any difference in their number. This strain did not maintain this quality for long, as after the second sub-inoculation from these rats the trypanosomes seemed to have become normal in their behaviour towards antimony. The strain was lost at this point.

Nagana Rats treated with Large Doses of Sodium Antimonyl Tartrate.

No.	Weight in grammes.	Total quantity of 1 per cent. sod. ant. tart. given, in c.c.	Recurrences.	Results.
1	200	5·75	1	Died on 30th day with intestinal lesions.
2	125	5·0	0	Died on 34th day with very marked intestinal lesions.
3	100	4·75	0	Died on 24th day: intestines necrotic.
4	100	4·0	0	Died on 108th day from pneumonia.
5	110	4·9	1	Died on 43rd day with inflamed intestines.
6	110	6·5	2	Died on 38th day with necrotic intestines.
7	175	8·5	2	Died on 40th day: trypanosomes in blood at death; paralysed.
8	110	9·0	2	Died on 45th day in similar condition to No. 7.
9	150	5·0	4	Died on 73rd day from gangrene of tail.
10	175	5·25	1	Died on 64th day from pneumonia.

Rats treated with Sodium Antimonyl Tartrate after Inoculation with Atoxyl-proof Trypanosomes.

The rats in the following table were all treated firstly with atoxyl to make sure that they were atoxyl-proof, and the treatment with antimony was then begun, when the trypanosomes were in large numbers in the blood.

	No.	Weight in grammes.	Total quantity of 1 per cent. sod. ant. tart. given, in c.c.	Recurrences.	Results.
Nagana…	1	100	3·0	1	Died on 29th day with trypanosomes in blood.
	2	100	1·0	0	Died on 11th day from nephritis.
	3	110	3·0	1	Died on 54th day.
	4	110	3·0	1	Died on 20th day with trypanosomes in blood.
	5	175	3·0	0	Died on 117th day from bronchopneumonia.
Surra……	6	100	3·0	0	Died on 27th day from septicæmia.
	7	145	5·75	1	Died on 41st day from pneumonia.
	8	140	4·0	1	Died on 38th day with trypanosomes in blood.

It will be seen that the atoxyl-proof strains of trypanosomes are less influenced by antimony than are the ordinary variety, as three of the above rats had living trypanosomes in the blood at death, and seven of them died at a very early date.

Rats treated with Antimony (Metal) and Sodium Antimonyl Tartrate suspended in a Fatty Medium.

Sodium antimonyl tartrate, when injected in watery solution into man produces very severe pain and inflammation in the neighbourhood of the injection, with more or less local necrosis. In order to make the use of antimony practicable in the form of injection, a series of experiments was undertaken, using various other media than water for solution or suspension of the antimony salt. Lanolin, olive oil, and sesamum oil were tried, but the results were not good. Finally, the medium Colonel Lambkin devised,* consisting of palmitin and antiseptics, which is used very largely for the intramuscular injection of calomel and mercury in syphilis, was tried, with the results which are set forth in the tables below.

One great advantage of these preparations is that they can be used upon man with far less difficulties and after-consequences than the watery solutions, which seem to be impracticable; this is of importance should antimony be found of use in human trypanosomiasis.

Major Ward, R.A.M.C., has used both the forms mentioned above on men for other purposes, and he has very kindly placed his notes at our disposal. In one of his cases four doses of ½ grain of sodium antimonyl tartrate, suspended in Colonel Lambkin's medium, were given intramuscularly into the buttock, the intervals between the doses being 3, 4, and 3 days: the doses were then increased to 1 grain, and seven doses of this strength were given, the intervals being 4, 3, 2, 4, 2, and 13 days. In all, this patient had 9 grains of the salt. Major Ward says that "the injections caused a certain amount of tenderness and discomfort at the seat of injection," but that is very different to the effects noticed after the injection of the watery solution.

Major Ward also treated two patients with antimony itself in a state of extremely fine division suspended in Colonel Lambkin's medium; they were each given one dose of 1 grain, and 11 days afterwards ½ grain. This form caused both pain and discomfort, and also a general increase in the size of the buttock, into which the injection was made, but this subsided without suppuration. This form would appear, however, to be much the more powerful of the two, as the effects obtained from the 1½ grains of the metal were as good and as lasting as those observed in the case in which 9 grains of sodium antimonyl tartrate were given.

The following tables show the results obtained in rats with sodium antimonyl tartrate and antimony prepared as mentioned above:—

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* 'Journ. Roy. Army Med. Corps,' 1906.

Sodium Antimonyl Tartrate, 5 per cent., in Colonel Lambkin's Medium.

	No.	Weight, in grammes.	No. of doses given.	Total quantity given, in minims.	Recurrences.	Results to August 20.
Nagana.	1	100	4	18	2	Died on 53rd day: bad itch.
	2	100	2	6	1	Died on 16th day with intestinal lesions.
	3	115	1	2	0	Died on 113th day of pneumonia.
	4	150	1	2	0	Died on 19th day of pneumonia.
	5	110	5	17	1	Died on 27th day: abscess.
	6	125	3	13	0	Died on 26th day of pneumonia.
	7	150	3	13	0	Died on 89th day: itch.
	8	175	1	3	1	Died on 24th day: abscess.
Surra.	9	200	3	17	1	Died on 40th day of injury.
	10	225	3	13	2	Died on 28th day with intestinal lesions.
	11	115	2	6	1	Died on 22nd day of pneumonia.
	12	220	11	33	7	Died on 66th day with acute intestinal lesions.
	13	125	1	3	0	*Living and well* 154 *days after inoculation.*

None of the above have died from trypanosomiasis. In rats the intramuscular injection of even a few minims is difficult, and if any of the material be left under the skin a slough forms, which takes a long time to heal. It will be noticed that No. 3 had only one dose: the rat lived 113 days and died of pneumonia; inoculations made from its organs were negative. No. 13, which is still living (154 days), has also had only one dose.

From the following table it will be seen that the administration of the metal itself has a considerable effect on the trypanosomes: it has a distinctly better effect on Surra than upon Nagana, four Surra rats out of 16 being still alive, and four others having lived for a long time. In none of the Surra rats were trypanosomes found at death, whereas in three of the Nagana rats they were present. The metal is much more irritating than the tartrate, but the effect is in most cases more prolonged; this is probably due to the fact that the absorption of the metal is much slower. Further, the smaller doses would appear to be the most efficient.

Antimony, in a state of very fine division, 5 per cent., in Colonel Lambkin's Medium.

	No.	Weight, in grammes.	No. of doses given.	Total quantity given, in minims.	Recurrences.	Results to August 20.
Nagana.	1	100	3	12	3	Died on 61st day : abscess.
	2	200	3	11	2	Died on 29th day with trypanosomes in blood.
	3	100	3	9	1	Died on 34th day with intestinal lesions.
	4	110	3	12	1	Died on 15th day with trypanosomes in blood.
	5	100	4	14	1	Died on 27th day with intestinal lesions.
	6	150	4	15	2	Died on 26th day with intestinal lesions.
	7	150	6	23	3	Died on 33rd day with trypanosomes in blood.
	8	300	3	13	4	Died on 51st day from broncho-pneumonia.
	9	300	2	8	2	Died on 36th day from broncho-pneumonia.
	10	200	2	8	0	*Living and well* 204 *days after inoculation.*
	11	300	2	8	1	Died on 29th day : abscess.
	12	250	2	8	1	Died on 39th day from broncho-pneumonia.
	13	250	2	8	7	Died on 92nd day from pneumonia.
	14	300	1	5	0	Died on 32nd day : fatty liver.
	15	300	1	5	0	Died on 30th day.
	16	150	1	3	0	Died on 74th day from pneumonia.
Surra.	17	110	1	5	0	*Living and well* 230 *days after inoculation.*
	18	125	4	17	4	Died on 146th day : one recurrence took place after an interval of 12 weeks.
	19	250	4	35	3	Died on 48th day from broncho-pneumonia.
	20	225	1	4	0	Died on 39th day from broncho-pneumonia.
	21	100	1	4	0	Died on 44th day : itch.
	22	100	3	10	1	Died on 44th day : itch.
	23	150	2	9	1	Died on 216th day of pneumonia.
	24	100	3	12	3	Died on 94th day : abscess. One recurrence took place after an interval of 46 days.
	25	100	2	9	0	*Living and well* 253 *days after inoculation.*
	26	150	2	9	0	Died on 29th day from pneumonia.
	27	125	1	3	0	*Living and well* 226 *days after inoculation.*
	28	100	1	3	0	Died on 91st day : itch.
	29	100	1	3	0	*Living and well* 226 *days after inoculation.*
	30	125	1	4	0	Died on 27th day from pneumonia.
	31	100	1	4	0	Died on 27th day from pneumonia.
	32	150	1	4	0	Died on 41st day : itch.

Experiments in which Antimony and Sodium Antimonyl Tartrate were given before Inoculation, in order to Test their Effects upon the Development of the Disease.

In these experiments the substances were given suspended in Colonel Lambkin's medium, and it will be noticed that the metal is far more effective in delaying the appearance of the trypanosomes in the blood than is the salt: this is probably due to the slower elimination of the metal. This method, if the doses were repeated, might be of some practical value in getting animals safely across dangerous tracts of country.

Four Rats were given 5 minims of 5 per cent. Antimony (metal) Cream on December 19. In Nagana the trypanosomes usually appear in the blood on the second or third day after inoculation.

No.	Inoculated with Nagana on—	Trypanosomes appeared in the blood on—
1	December 20......	December 31, the 11th day.
2	,, 21......	January 2, the 10th day.
3	,, 23......	December 31, the 8th day.
4	,, 24......	,, 30, the 6th day.

Four Rats were given 3 minims of 5 per cent. Sodium Antimonyl Tartrate Cream on December 19.

No.	Inoculated with Nagana on—	Trypanosomes appeared in the blood on—
1	December 20......	December 25, the 5th day.
2	,, 21......	,, 24, the 3rd day.
3	,, 23......	,, 26, the 3rd day.
4	,, 24......	,, 27, the 3rd day.

Rats treated with Lithium Antimonyl Tartrate.

There are differences in the effects produced by the potassium, sodium, and lithium antimonyl tartrates, if given under similar conditions and dosage. The commercial potassium salt is very impure. The pure sodium and lithium salts which we have used have been prepared for us by Dr. R. H. Aders Plimmer, of University College. The sodium salt contains roughly about 2 per cent. more antimony than the potassium salt, and the lithium salt contains about 2 per cent. more antimony than the sodium; but the doses of the lithium salt have to be much smaller than the corresponding doses of the sodium salt. For instance, 0·5 c.c. of a 1 per cent. solution of the lithium salt is fatal to a rat of 125 grammes, and 0·39 c.c. is fatal to a rat of 80 grammes. When the watery solution is injected intramuscularly, it has not caused necrosis of the tissues in rats, but subcutaneously it has occasionally done so. We have found that the best strength of solution for rats is 0·25 per cent., and of this 0·5 c.c. has been given for a dose.

The following table shows the effects of this dosage.

	No.	Total quantity in c.c. given of 0·25 per cent. solution.	Recurrences.	Results to August 20.
Nagana...	1	7	2	Died of disease on 47th day.
	2	7	1	Killed on 47th day, owing to abscess. Emulsion of organs injected into another rat gave negative result.
	3	4·5	1	Allowed to die on 34th day, the date of recurrence.
	4	4·5	0	*Alive and well* 134 *days after inoculation.*
	5	4	0	*Alive and well* 125 *days after inoculation.*
Surra......	6	4	0	*Alive and well* 134 *days after inoculation.*
	7	4	0	Died on 42nd day of broncho-pneumonia: no evidence of trypanosomes.
	8	4	0	*Alive and well* 134 *days after inoculation.*
	9	5	2	Died of disease on 47th day.
	10	4·5	0	*Alive and well* 125 *days after inoculation.*
	11	3·5	2	Died of disease on 18th day: itch.
	12	3	1	Allowed to die: relapse not treated.

From the above it will be seen that five out of these 12 rats are alive and well at periods varying from 125 to 134 days. This salt is much more soluble than either the potassium or sodium compound, which may, perhaps, as well as its greater antimony content, account for its greater effectiveness. It is, however, more irritating subcutaneously in watery solution.

Experiments with Antimony upon Dogs.

In order to see what the effects of antimony would be on the larger and more important animals when suffering from trypanosomiasis, a series of experiments on dogs has been begun. The trypanosome used was that of Surra, which kills dogs of about 20 lbs. in weight in approximately 14 days, as this is the trypanosome which is of practical importance with regard to dogs.

Dr. MacConkey, of the Lister Institute, kindly performed some initial experiments for us. He made some experiments with a 20 per cent.

suspension of sodium antimony tartrate in Col. Lambkin's medium, in order to find the minimum lethal dose, and he found that 0·5 c.c. of this 20 per cent. cream per 10 lbs. of body weight was probably about the full dose. But he also found that, for practical purposes, this 20 per cent. cream was much too strong, as it caused sloughing in every case; indeed, dogs do not seem to bear these suspensions as well as they do solutions. Dr. MacConkey kept one dog alive till the 40th day, and one until the 30th, but both had many relapses.

Profiting by the experience thus gained, we have made further experiments using much smaller doses, with satisfactory results. But we have found, since trying the lithium antimonyl tartrate that this acts more effectually and with less irritation than the creams, whether of metal or salt. All the animals tabulated below are in good condition and are gaining in weight.

(Average length of untreated disease, 14 days.)

No.	Weight, in lbs.	Total quantities given, in minims.	Recurrences.	Results to August 20.
1	22	Antimony cream, 5 per cent., 60 m. ...... Sod. ant. tart., 2 per cent., 40 m.	2	No trypanosomes present since July 13; is alive and well on the 62nd day after inoculation.
2	22	Sod. ant. tart., 2 per cent., 80 m. .... Lith. ant. tart., 2 per cent., 55 m.	2	Had 9 pups during treatment; there were 42 days between the two recurrences; is alive and well on the 62nd day after inoculation.
3	37½	Sod. ant. tart. cream, 5 per cent., 80 m. Sod. ant. tart., 2 per cent., 40 m. Lith. ant. tart., 2 per cent., 90 m.	6	Incorrect dosage seems a probable cause of these relapses; is alive and well on the 62nd day after inoculation.
4	16	Sod. ant. tart. cream, 5 per cent., 40 m. Sod. ant. tart., 2 per cent., 20 m. Lith. ant. tart., 2 per cent., 40 m.	3	Is alive and well on the 53rd day after inoculation.
5	13½	Sod. ant. tart. cream, 5 per cent., 35 m. Lith. ant. tart., 2 per cent., 47 m.	3	Is alive and well on the 53rd day after inoculation.

The second and third substances mentioned in Column 3 were given at the recurrences. That these dogs are all alive and well encourages us to hope that, as we get a better knowledge of the dosage required, the recurrences may be less frequent.

Experiments made with Rats treated with Antimony, in order to find out in what Organs the Trypanosomes are latent.

The following initial experiments were made in order to find out where the trypanosomes rest during the period in which the peripheral blood is free from them, after treatment with antimony. Further experiments are being carried on for the purpose of ascertaining in what forms they are present in the organs of treated animals.

The rats were inoculated with Nagana, which is less affected by antimony than Surra, and were all treated with four doses of sodium antimony tartrate. The rats were killed at various intervals, and the organs selected (the liver and bone-marrow) were made into an emulsion with a minimum quantity of 0·75 per cent. salt-solution, and injected into other rats in doses of 1 c.c.; the same dose of blood from the heart was also given.

In the following table the signs + and − are used respectively to denote a positive or negative result.

No.	Number of days after treatment upon which rats were killed.	Blood.	Liver.	Marrow.
1	7	−	−	−
2	10	−	−	−
3	12	−	−	−
4	14	−	−	−
5	2	−	+	+
6	4	−	+	−
7	3	−	+	+
8	12	−	−	+
9	14	−	−	+
10	16	−	−	+
11	20	−	−	+

From this table it would appear that the bone-marrow is the place where the trypanosomes can live longest, and that the liver is also a place where they can find protection. This is borne out by some experiments we have made upon trypanosomiasis in birds, in which cultivations of trypanosomes can often be made from the bone-marrow when they cannot be made either from the organs or the blood. The doses given to the above rats were rather under those which we should judge to be curative, but in four cases the results were entirely negative.

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ALVEOLAR AIR ON MONTE ROSA.

By R. OGIER WARD, B.A., Oxford[1].

THE object of these experiments was to compare the effects upon the composition of alveolar air, produced by living at great altitudes, with such variations as are obtained by artificially altering the barometric pressure, with special reference to experiments made by Haldane and Boycott at the Lister Institute, in some of which I had the opportunity of taking part (see preceding paper).

All the determinations were made by the method of Haldane and Priestley and with the Haldane gas analysis apparatus.

A fortnight was devoted to determining the normal tension of carbonic acid in R. O. W.'s alveolar air under ordinary barometric pressures in London.

It was found to be:—

Bar.	Mean CO_2 %	Tension of CO_2 mms. Hg.	No. of exps.	Max. variation
769	5·22	37·7	20	40·5 to 35·8 mms. Hg.

The oxygen was only determined in a few cases and was found to be:

Mean O_2 %	Tension of O_2 mms. Hg.
15·12	109·0

values which agree closely with those calculated from the CO_2:

Mean O_2 %	Tension of O_2 mms. Hg.
14·79	106·9

Further experiments made more recently have confirmed these results.

At the same time the normal hæmoglobin was determined with a standardized Gowers' hæmoglobinometer, and the normal blood-pressure with Martin's modification of the Riva Rocci apparatus. These were found to be:—Subject, R. O. W.; Hæmoglobin 101; Blood-Pressure 109 mm.

On July 25th, 1907, a move was made from London to Switzerland and a week was spent at Zermatt (alt. 5315 ft.), during which time the experiments were continued. Table I gives the results:—

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[1] Nominated by the Royal Society to the International Laboratory on Monte Rosa.

TABLE I.

At Hotel Monte Rosa, Zermatt. (Altitude 5315 feet.) Subject: R. O. Ward.

		Barometer	Room temp.	CO_2 end of expiration	CO_2 end of inspiration	Mean CO_2 per cent.	Mean CO_2 mm. Hg.	O end of expiration	O end of inspiration	Mean O_2 per cent.	Mean O_2 mm. Hg.	Hæmoglobin	Blood-pressure	Exercise (climbing)
July 27	6.15 p.m.	633·5	18°	6·20	5·76	5·98	35·1	12·82	13·33	13·07	76·8	—	—	5 hrs.
	7.45 p.m.		16	6·15	5·79	5·97	35·0	13·01	13·86	13·43	78·9	—	—	
28	10.0 a.m.	636·5	15	5·87	5·69	5·78	34·1	13·98	14·44	14·21	83·9	—	—	
	10.45 a.m.		16	5·84	5·52	5·68	33·5	13·88	14·70	14·29	84·4	101	101	
	6.30 p.m.	635·5	18	5·97	5·78	5·87	34·6	13·30	13·77	13·53	79·8	98	—	
	8.20 p.m.		17	5·92	5·88	5·90	34·7	13·91	13·96	13·93	82·1	—	—	
29	9.45 a.m.		15	6·35	6·12	6·23	36·7	13·34	14·06	13·70	80·8	106	—	5 hrs.
	6.5 p.m.	632·5	18	5·48	5·60	5·54	32·5	14·17	13·84	14·00	82·1	—	—	
	6.30 p.m.		18	5·68	5·61	5·64	33·1	13·65	13·93	13·79	80·9	—	—	
	6.55 p.m.		18	5·72	5·56	5·64	33·1	—	—	—	—	—	113	
30	9.45 a.m.	628·5	17	5·95	5·78	5·87	34·2	14·69	14·45	14·57	84·9	102	—	
	11.5 p.m.		17	5·90	5·47	5·68	32·9	14·01	15·07	14·54	84·7	—	—	
31	3.10 p.m.	627·5	18	6·34	6·13	6·23	36·2	13·60	13·84	13·72	79·8	—	106	6 hrs.
Aug. 2	3.10 p.m.	632·5	18	5·84	5·62	5·73	33·6	—	—	—	—	—	104	
	Mean	633	17			5·84	34·2			13·90	81·6			

It will be seen that although the CO_2 percentage has risen slightly, the fall of barometric pressure from 769 mms. to 633 mms. has caused a considerable drop in the partial pressure of CO_2, which is now 34·2 mms. as against a normal of 37·7 in London. The oxygen percentage has also fallen from a normal 15·12 % (109 mms. Hg) to 13·90 % (81 mms.).

The first experiment was made within about 27 hours of arrival, and the change seems to have been complete by this time, since the figures for the following days show only slight irregularities. There has been no change in the amount of hæmoglobin, and the blood-pressures continue normal.

The exercise taken was almost entirely in the form of climbing; the total time for each expedition is given in the table.

On August 2nd R. O. W., W. D. H., G. A. H. with guides and porters moved towards Monte Rosa; the night was spent at the Bétemps Hut (alt. 8860 ft.), an early start was made next morning and the Capanna Regina Margherita on the summit (alt. 14,965 ft.) was reached at 7.55 a.m. after 6 hrs. of somewhat laborious ascent. R. O. W. and W. D. H. remained at the hut, whilst G. A. H. descended.

The next table gives the results of experiments on R. O. W. made during the seven days spent on the summit. Exercise consisted in the ascents of the surrounding peaks.

It will be noticed that although the CO_2 percentage has risen from a mean of 5·84 % at Zermatt (Table I) to 7·18 %, the lowered barometric pressure has caused the CO_2 tension to fall from 34·2 at Zermatt to 28·5 mms., and the oxygen tension from 81·6 mms. (Table I) to 49·8 mms.

In the first experiment, made three hours after arrival, the CO_2 tension is 30·6 mms., only slightly above the mean for the succeeding days, and since this same value occurs on August 5th and 6th all change seems to have been complete in the first few hours and the table cannot be said to show any continued adaptation to the altered conditions of pressure.

On August 3rd, the day of arrival, R. O. W. experienced in a slight degree symptoms, such as shortness of breath, headache, chilliness, which may be attributed to the effect of altitude; these however passed off, but were succeeded on the following day by a severe bilious attack which came on during the night and lasted twenty-four hours. Reference to Table II will show that this disturbance, which undoubtedly was a variety of mountain sickness, is not represented in the figures, since in the first experiment of the day (Aug. 4th, 9.25 a.m.), which was made during the

TABLE II.

At Capanna Regina Margherita, Monte Rosa. (Altitude 14,965 feet.) Arrived 8 a.m. 3rd Aug. Subject R. O. Ward.

				CO$_2$ per cent.				Oxygen per cent.						
		Barometer	Room temp.	Expiration	Inspiration	Mean	Mean CO$_2$ mm.	Expiration	Inspiration	Mean	Mean O$_2$ mm.	Hæmoglobin	Blood-pressure	Exercise (climbing) and Remarks
Aug. 3	11.0 a.m.	442	6°	7·68	7·47	7·58	30·0	11·60	11·50	11·55	45·7	—	—	6 hrs. to reach hut.
	6.45 p.m.		7	7·47	7·17	7·32	29·0	12·03	11·87	11·95	47·3	107	106	
4	9.25 a.m.	444	14	7·11	6·55	6·88	27·4	11·57	12·56	12·06	48·0	—	—	(Bilious).
	6.55 p.m.	444	8	6·81	6·66	6·73	26·8	11·13	12·40	11·76	46·8	107	105	
5	10.45 a.m.	444	8·5	7·54	7·24	7·39	29·4	12·96	12·74	12·85	51·2	—	—	
	11.30 a.m.			7·45	7·26	7·35	29·2	12·22	12·59	12·40	49·3	111	102	2½ hrs.
	8.45 p.m.		11	7·70	7·35	7·52	30·0	11·57	12·32	11·94	47·5	—	—	
6	9.15 a.m.	443	1·5	7·65	7·50	7·57	30·0	12·03	12·15	12·09	48·0	—	—	
	6.20 p.m.		10	7·48	7·48	7·48	29·7	12·55	12·71	12·63	50·1	110	106	
7	9.30 a.m.	441	7	7·31	7·15	7·23	28·5	12·74	13·06	12·90	51·0	—	—	
	10.15 a.m.		8	7·35	7·09	7·22	28·5	12·80	13·24	13·02	51·4	—	—	
	6.40 p.m.		6	7·35	7·20	7·27	28·7	12·45	12·50	12·47	49·3	111	108	
8	9·20 a.m.	444	1	6·98	6·57	6·77	26·9	13·38	14·09	13·73	54·6	—	—	
	10.0 a.m.		3	7·09	6·93	7·01	27·9	13·01	13·19	13·10	52·1	—	—	2¼ hrs.
	6.10 p.m.	445	6	7·19	7·42	7·30	29·1	12·27	12·97	12·62	50·4	113	107	
9	8.35 a.m.	444	7	6·93	6·70	6·81	27·1	12·74	13·37	13·05	51·9	—	—	
	8.55 a.m.			6·84	6·75	6·79	27·0	13·30	13·34	13·32	53·0	—	—	
	3.0 p.m.	445	10	7·04	7·07	7·05	28·1	12·17	12·74	12·45	49·7	115	110	2½ hrs.
	Mean	443·5	7			7·18	28·5			12·55	49·8			

most severe part of the attack, the CO_2 tension is 27·4 mms., and this is not so low as that obtained on Aug. 9th: also the O_2 tension, 48·0 mms., is repeated two days later. From this it would seen that an adaptation to maintain the supply of oxygen which was sufficient to keep R. O. W. well during the rest of the week in the hut, was however not enough to prevent an attack of mountain sickness. Similar results will be seen in the case of W. D. H.

The amount of hæmoglobin in the blood found on the day of arrival is in excess of that obtained at Zermatt, and this rise continued slowly during the week, reaching 115 on August 9th. This may be connected with the somewhat higher percentages of oxygen shown in the latter part of the table. For short periods, however, this secondary compensation cannot be of much importance, being overshadowed by the increasing falling off of bodily fitness, a fact which was appreciable even at this altitude.

Whenever expeditions were made R. O. W. and W. D. H. always experienced the same shortness of breath during the ascent of the last 200 feet to the hut as on the first occasion. These considerations in conjunction with the fact that the tables show no continued improvement in the composition of alveolar air, support the opinion that during the ascent of a big mountain it is inadvisable to stay longer than necessary at a high camp (E. A. Fitzgerald, T. G. Longstaff). It will be seen that the blood-pressures remained quite normal, which may be taken as an indication of general fair condition.

On August 9th G. A. H. reached the hut with guide and porter, and the following morning at 7.45 a.m. the descent was begun, the party was off the snow by 10.0 a.m., and to avoid fatigue as much as possible the rest of the journey was not hurried, Zermatt being reached at about 3.30 p.m. Table III gives the results of experiments made during the next week.

It will be seen at once that a return to higher barometric pressures has not restored the composition of alveolar air to the normal previously found for Zermatt.

To consider the first determination (6.40 p.m.); the CO_2 percentage is now 4·90 $^{0}/_{0}$, whereas the mean obtained in Table I was 5·84 $^{0}/_{0}$; this has caused the CO_2 tension to fall, this being now 28·7 instead of 34·2. As a result of this low CO_2 percentage the oxygen percentage has risen, being now 15·51 $^{0}/_{0}$ as against 13·90 $^{0}/_{0}$ in Table I, thus giving an oxygen tension of 91·0 mms. which is 9·4 mms. above what was previously determined to be the normal for Zermatt, 81·6 mms. In short the

TABLE III.

At Zermatt, after return from Capanna Regina Margherita. Subject: R. O. Ward.

		Barometer	Room temp.	CO_2 end of expiration	CO_2 end of inspiration	Mean CO_2 per cent.	Mean CO_2 mm. Hg.	O_2 end of expiration	O_2 end of inspiration	Mean O_2 per cent.	Mean O_2 mm. Hg.	Hæmoglobin	Blood-pressure	Exercise (climbing)
Aug. 10	6.40 p.m.	632·5	15°	4·87	4·93	4·90	28·7	15·72	15·31	15·51	91·0	113	—	Descent from Monte Rosa, 4 hrs.
	6.50 p.m.			5·16	4·76	4·96	29·1	—	—	—	—	—	—	
	8.15 p.m.		14	5·73	5·31	5·52	32·4	—	—	—	—	—	—	
	8.30 p.m.			5·65	5·45	5·55	32·6	13·97	14·37	14·17	83·1	—	105	
11	9.45 a.m.	634·5	15	5·20	4·98	5·09	29·9	14·77	15·45	15·11	88·9	—	—	
	10.15 a.m.			5·19	5·05	5·12	30·1	15·06	15·37	15·21	89·5	—	—	
	5.10 p.m.		18	5·24	5·00	5·12	30·1	15·42	15·66	15·54	91·4	109	—	
12	10.25 a.m.	634·5	17	5·36	5·16	5·26	30·9	—	—	—	—	—	—	5 hrs. hard walking.
13				—	—	—	—	—	—	—	—	—	—	12½ hrs.
14	11.50 a.m.	635·5	19	4·94	4·84	4·89	28·8	15·90	15·89	15·89	93·7	—	—	
	12.0			4·93	4·69	4·81	28·3	—	—	—	—	—	—	
	6.15 p.m.	634·5		5·08	5·07	5·07	29·8	15·43	15·34	15·38	90·5	—	—	
15	9.40 a.m.	631·5	18	5·38	5·35	5·36	31·4	15·63	15·60	15·61	91·4	105	—	3¼ hrs. hard walking.
	6·40 p.m.	628·5	17	5·58	5·17	5·37	31·3	14·99	15·17	15·08	87·8	—	—	
16	10.25 a.m.	627·5	17	5·78	5·40	5·59	32·5	14·77	15·56	15·16	88·1	—	—	
	4.0 p.m.			5·74	5·46	5·60	32·6	14·83	15·15	14·99	87·2	—	—	

alveolar air now contains an abnormally small amount of CO_2 and an excessive amount of oxygen.

A second experiment made immediately afterwards (6.50 p.m.) will be seen to support these figures.

It should be noticed that the CO_2 tension in the first experiment is almost exactly the same as the normal found for Monte Rosa, a point which will be discussed later.

Two more experiments were made the same night; in these the percentages of CO_2 and oxygen have varied towards the normal. On the next day, however, the CO_2 percentages and tensions are again low, though showing a tendency to rise, which continues till August 12th.

It will be noticed that on August 14th the results of the three experiments once more resemble the first determinations made after the descent. This may perhaps be explained by the fact that the night of August 12th was spent in the Weisshorn hut (alt. 9380 ft.) and on the morning of the 13th the mountain itself was ascended (alt. 14,804 ft.), the altitude probably encouraging a return to the conditions produced by the stay on Monte Rosa.

From this date onwards a continuous rise of CO_2 and fall of oxygen percentage will be seen, though in seven days after descending from Monte Rosa the normal for Zermatt had not been reached.

No further experiments were made till November: the results obtained then in Oxford will be seen to agree with the normal as found in London in July.

	Bar.	Mean CO_2 %	Tension of CO_2 mms. Hg.
	752	5·33	37·6
	752	5·31	37·5
	752	5·30	37·4
	753	5·42	38·3
	753	5·33	37·7
Means:	752	5·34	37·7

Table III also shows a slow fall in the amount of hæmoglobin which, however, was still high after six days at Zermatt. It also gives a normal blood-pressure obtained on the evening of the descent.

Table IV gives the experiments made on W. D. H. who was with R. O. W. at Zermatt and on Monte Rosa.

Whilst these figures resemble in their chief features those obtained in the case of R. O. W. yet a comparison of this table with Table II will show that the favourable variations between Zermatt and Monte

TABLE IV.

Subject: W. D. Hutchinson.

		Barometer	Room temp.	CO_2 end of expiration	CO_2 end of inspiration	Mean CO_2 per cent.	Mean CO_2 mm. Hg.	O_2 end of expiration	O_2 end of inspiration	Mean O_2 per cent.	Mean O_2 mm. Hg.	
At Zermatt:—												
July 30	11.45 p.m.	626·5	17°	6·19	5·83	6·01	34·9	—	—	—	—	
31	3.30 p.m.	627·5	18	6·23	6·01	6·12	35·6	—	—	—	—	
At Capanna Regina Margherita:—												
Aug. 3	8.0 p.m.	442	7	8·60	7·63	8·11	32·1	10·31	12·02	11·16	44·2	W. D. H. was sick during the night Aug. 3–4. Exercise the same as R. O. W.
4	2.20 p.m.	444	6	8·36	7·77	8·06	32·1	10·82	11·68	11·25	44·8	
5	6.0 p.m.	444	9·5	8·26	7·99	8·13	32·3	10·07	10·61	10·34	41·2	
6	3.35 p.m.	443	13	8·59	7·85	8·22	32·6	11·21	11·96	11·58	46·0	
7	3.35 p.m.	442	8	8·31	7·67	7·99	31·6	11·18	12·42	11·80	46·7	
8	5.50 p.m.	445	5	8·11	7·85	7·98	31·8	11·02	11·47	11·24	44·9	
9	2.15 p.m.	445	9	7·97	7·68	7·82	31·2	10·99	11·54	11·26	44·9	
Mean		444	8			8·04	31·9			11·23	44·7	
At Zermatt:—												
Aug. 10	8.50 p.m.	632·5	14	5·78	5·71	5·74	33·7	14·07	13·06	13·57	79·6	
	9.10 p.m.			5·87	5·69	5·78	33·9	—	—	—	—	
11	5.50 p.m.	634·5	18	6·04	5·91	5·97	35·1	14·02	14·43	14·22	83·7	

Rosa are here not so marked. Thus the CO_2 percentage has changed from about 6·12 °/₀ at Zermatt to a mean of 8·04 °/₀, a rise of 1·92, whereas in the case of R. O. W. there was only a rise of 1·34 in the percentage of CO_2. Consequently the fall in CO_2 tension is also less, being only about 3·7 mms. for W. D. H. as against a drop of 5·7 mms. for R. O. W. The important result of this alteration is that whereas at Monte Rosa R. O. W. had a mean oxygen percentage of 12·55 °/₀ (49 mms.), for W. D. H. the percentage is only 11·23 °/₀ and the tension 44·7 mms.: that is he had 11 °/₀ less oxygen in his alveolar air than R. O. W.

It will also be seen from the table that in this case also on returning to Zermatt the alveolar air did not at once regain its previous composition, as indicated by the CO_2 tension.

As regards symptoms, those of W. D. H. were on the whole more severe than those of R. O. W. He suffered from mountain sickness more acutely. During the first night at Monte Rosa he was sick three times, on each occasion waking up with a sense of suffocation; and several times in the evenings of the first two or three days at the hut he was observed to be slightly cyanosed when sitting still. R. O. W. on the other hand was only troubled by the bilious attack mentioned. It may be added that W. D. H. was undoubtedly the better physically trained of the two. The exercise taken was the same for both.

TABLE V.

Subject: G. A. Hutchinson.

		CO_2 end of expiration	CO_2 end of inspiration	Mean CO_2 per cent.	Mean CO_2 mm. Hg.
At Zermatt :—					
July 30	11.25 p.m.	5·85	5·93	5·89	34·1
	11.35 p.m.	6·10	5·93	6·01	34·9
31	3.45 p.m.	6·18	5·92	6·05	35·2
At Capanna Regina Margherita :—					
Aug. 3*	8.30 a.m.	7·79	7·38	7·58	30·0
9	1.30 p.m.	7·85	7·51	7·68	30·6
	1.40 p.m.	8·06	7·74	7·90	31·5
At Zermatt :—					
Aug. 10	6.25 p.m.	5·58	5·12	5·35	31·4
11	6.25 p.m.	5·42	5·07	5·24	30·8
14	12.15 p.m.	5·49	5·29	5·39	31·8

* Descended the same morning to the Riffelhaus. On Aug. 9th ascended to the Margherita Hut, climbing the Lyskamm on the way, and arriving at 11.30 a.m.

Table V gives the results of experiments made on G. A. H. who accompanied R. O. W. and W. D. H. to Monte Rosa. He went down the same morning, returning on August 9th to assist in the descent next day.

The changes in the CO_2 tension, which will be seen to fall from about 34·9 mm. to about 30·6 mm., are more marked than in the case of W. D. H. and resemble those for R. O. W.

The following is a summary of results :

TABLE VI.

Subject : R. O. W.

				Some experiments at Lister Institute.	
	Bar.	Tension of CO_2 mms. Hg.		Bar.	Tension of CO_2 mms. Hg.
London (mean)	769	37·7		640	36·6
Zermatt (mean)	633	34·2		640	37·5
				619	36·6
				621	37·1
				621	37·5
			Means	628	37·1
Monte Rosa (mean)	443·5	28·5		423	35·3
				458	35·6
				458	35·8
				495	36·0
				495	35·9
				540	36·4
				541	36·4
				541	35·9
			Means	494	35·9

Table VI makes it clear that the composition of alveolar air is very differently affected by short experimental diminution of barometric pressure, and by an actual ascent to an equivalent height.

Thus whilst it will be seen that as a result of a move from London to Zermatt the CO_2 tension falls from a mean of 37·7 to 34·2 mms., it will also be seen that in the short experiments at the Lister Institute, a lowering of the barometric pressure to 628 mms., more than equivalent to the height of Zermatt, only caused the tension to fall to 37·1 mms. Furthermore under a barometric pressure reduced experimentally to 494 mms., not only is the CO_2 tension (now 35·9) far greater than the mean for Monte Rosa, but it has not even fallen to the figure found for Zermatt.

The chief point of difference between the two sets of experiments is doubtless the length of time given to the ascent.

It would seem that under a short experimental lowering of barometric pressure the organism does not have time to complete its reaction to altered conditions. How long may be required for this the experiments do not show, but that, for a rise of 9650 ft. from Zermatt to Monte Rosa, it cannot be more than 20 hours, is proved by the fact that, in the case of R. O. W., only this time elapsed between the last experiment at Zermatt and the first at Monte Rosa; and reference to Table II shows that no further definite alteration was observed. It must be remembered, however, that here the change may have been accelerated by the effort of climbing. The lower room temperatures at the Capanna Regina Margherita cannot afford an explanation of these differences, since the usual effect of cold upon the alveolar air is to raise the CO_2 tension.

At least one form of adaptation is shown by these experiments. If at an altitude of 14,965 ft. the CO_2 tension continued at that figure which is constant for sea level, for R. O. W. 37·7 mms., then the percentage of CO_2 in the alveolar air would be 9·5 %, a condition which would give an oxygen percentage of about 9·8 %, thus producing an oxygen tension as low as 39·0 mms.

As we have seen, the CO_2 tension actually falls so that the percentage of CO_2 does not reach this high figure, and in this way the oxygen percentage and tension are kept up at a level more approaching the normal for ordinary barometric pressures; thus on Monte Rosa in the case of R. O. W. the oxygen percentage only dropped to 12·55 % and the tension to 49·8 mms.

A comparison of Tables II and IV will show that the figures fully account for the fact that W. D. H. suffered most discomfort while on Monte Rosa, although of the two he was distinctly in better physical condition, for, as has been pointed out, his alveolar air always contained less oxygen than that of R. O. W.

Since the observations on Monte Rosa were made, a long experiment under low pressure has been carried out in the steel chamber at the Lister Institute, with myself as the subject. The results are shown graphically in Fig. 5 of the preceding paper by Boycott and Haldane. This experiment shows clearly that there was a continuous fall in the alveolar CO_2 tension during the first two or three hours of exposure to the lower pressure, and that 20 hours later there was only a slight further increase in this fall: also that on returning to normal barometric pressure it took from one to two days for the alveolar CO_2 tension to return to its normal value. Long exposure in the chamber thus gives results very similar to those observed at Zermatt and Monte Rosa. In the chamber at 540 mm. air-pressure the fall in alveolar CO_2 pressure was about 6 mm., as compared with about 9 mm. on Monte Rosa at 447 mm. air-pressure. Allowing for the difference in air-pressure, the effect in the chamber seemed, therefore, to be fully as great as on the mountains, if not greater. It was remarkable that in spite of very free ventilation, comfortable accommodation, and absence of muscular fatigue, nausea and sleeplessness were produced in the chamber with a fall of pressure to only 540 mm. We had purposely not gone further, with a view to avoiding mountain sickness; but probably this was brought on more readily in the chamber on account of the far more rapid fall in pressure than occurs in mounting climbing.

It is doubtless a fortunate circumstance for mountaineers that the effects on respiration of exposure to high altitudes take so long to pass off: for the consequence is that the beneficial effects of descending are very promptly felt. At a given altitude on the descent the alveolar oxygen pressure will probably be higher than at the same altitude on the ascent, with corresponding relief to the symptoms.

A further general discussion of the results is contained in the preceding paper by Boycott and Haldane.

The writer expresses his sincere thanks to Professor A. Mosso who kindly allowed him the use of the laboratory in the Capanna Regina Margherita, and also to the Director of the Lister Institute of Preventive Medicine.

The gas analysis apparatus was kindly lent by Professor Gotch.

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A NOTE ON THE VARIATION OF THE RATE OF DISINFECTION WITH CHANGE IN THE CONCENTRATION OF THE DISINFECTANT.

By HERBERT EDMESTON WATSON, B.Sc. (Lond.), A.I.C.

In a recent paper by Miss Chick on "The Laws of Disinfection[1]," it was pointed out that disinfection of bacteria is strictly analogous to a chemical reaction in which individual bacteria play the part of molecules. Thus, if n be the number of bacteria present at any time t during disinfection, $-\frac{dn}{dt} = K \, . \, n$, where K is a constant. Also, if K_1, K_2 are these constants for two different temperatures T_1, T_2, $\frac{T_1 T_2}{T_1 - T_2} \log \frac{K_1}{K_2}$ is also constant, *i.e.* Arrhenius' formula for the temperature coefficient of chemical reactions holds good in the case of bacteria as well. In addition to this, it was found that the relation between the concentration of the disinfectant and the time of disinfection (that is, the time required to reduce the original number of bacteria by a given percentage) might be approximately expressed by the empirical law

$$\frac{1}{C_0 - C} \log \frac{Ct}{C_0 t_0} = \text{a constant},$$

where C is the concentration at time t.

Now it is at once evident that this last expression admits of no physical interpretation, whereas the two former ones may be deduced theoretically, and the meaning of all the constants in them is intelligible. Consequently it seemed probable that some other expression with a physical meaning might be found which would replace the empirical one, and by analogy with chemical reactions it was thought that a law of the form $c^n . t =$ a constant, might hold, t being the time of disinfection for concentration c of disinfectant, and n being a constant, for it may be shown that this is the relation when one molecule of one substance reacts with n molecules of a second, the latter being in great excess.

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[1] This *Journal*, vol. VIII. p. 92, 1908.

For purposes of calculation, this law may be written

$$n \,.\, \log c + \log t = \text{constant},$$

that is to say, the relation between $\log c$ and $\log t$, is a linear one.

The formula was applied to Miss Chick's results, making use of a graphical method in which $\log c$ was plotted against $\log t$. In every case the result was a straight line from the slope of which n could be calculated[1].

The following tables show the nature of the constant obtained when this value of n was substituted in the formula $n \,.\, \log \,.\, c + \log \,.\, t = \text{const.}$ The first two columns give the relative concentrations and the times of disinfection, and are taken from the most suitable experiments recorded in Miss Chick's paper. Also for the sake of comparison, the values of the constant K obtained by means of the empirical formula $\frac{1}{C_0 - C} \log \frac{Ct}{C_0 t_0}$ are given in the third column.

TABLE I.

Disinfection of B. paratyphosus by phenol. 20° C.

Parts phenol per 1000	Time taken for disinfection	'K'	$5{\cdot}5 \log c + \log t$
8	45 minutes	—	6·62
7·5	75	0·39	6·69
7	105	0·31	6·67
6·5	125	0·24	6·58
6	225	0·29	6·64
5·5	440	0·29	6·71
5	690	0·33	6·68

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[1] It may perhaps be here mentioned that in cases of this kind, a graphical method is greatly superior to the usual methods of calculation, provided that the quantities plotted are so chosen that the resulting curve extends well across the paper, and, if possible, approximates to a straight line. Actually in the present case, the advantage is not very great, but in formulae such as $K = \frac{1}{t - t_0} \log \frac{n_0}{n}$, employed by Madsen and Nyman, *Zeitschr. f. Hygiene*, vol. LVII. p. 388, 1907, and H. Chick, this *Journal*, vol. VIII. p. 92, 1908, to express the reaction velocity of disinfection, the calculated value of K may lead to quite erroneous results, firstly because the values t and n may be inaccurate if taken from a single experimental value, as is usually the practice, and secondly, because the effect of a given experimental error on the value of K is greatly exaggerated when $t - t_0$ is small, while the same error when $t - t_0$ is great is almost inappreciable. The magnitude of these errors is, however, at once seen from a suitably drawn curve. Consequently, before making any deductions from values calculated by means of a formula, it is always advisable to examine the graphical solution to see if they are justified.

TABLE II.

Disinfection of Staph. pyogenes aureus by phenol. 20° C.

Parts phenol per 1000	Time taken for disinfection	'K'	$5{\cdot}5 \log c + \log t$
14	4·5 minutes	—	6·95
12·5	2·5	—	6·42
10	25	0·15	6·90
8	95	0·16	6·94
7	186	0·20	6·92
6	395	0·20	6·88
4	1425	0·19	6·47

TABLE III.

Disinfection of Anthrax spores by mercuric chloride. 18° C.
(Krönig and Paul.)

Relative concentration of Hg^{++} ions	Time taken for disinfection	'K'	$4{\cdot}9 \log c + \log t$
88·5	4·54 minutes	—	10·20
83·0	7·14	0·031	10·26
76·8	12·5	0·032	10·35
69·0	14·4	0·023	10·19
61·0	38·4	0·028	10·33

TABLE IV.

Disinfection of B. paratyphosus by mercuric chloride. 20° C.

Relative concentration of Hg^{++} ions	Time taken for disinfection	'K'	$3{\cdot}8 \log c + \log t$
63	1·5 minutes	—	7·02
57·5	7	—	7·54
42·5	13	0·037	7·31
37	10	0·023	6·95
23	65	0·030	6·99
16·5	230	0·027	6·98

TABLE V.

Disinfection of B. paratyphosus by silver nitrate. 20° C.

Concentration of $AgNO_3$	Time taken for disinfection	'K'	$0{\cdot}86 \log c + \log t$
5000	0·75 minutes	—	3·06
1000	1·5	0·10	2·76
500	2·5	0·11	2·70
100	6·5	0·15	2·53
50	22·5	0·10	2·81
10	56	0·16	2·61
5	140	0·14	2·75
1	>390	—	>2·59

From these figures it will readily be seen that the constant obtained is as good as may be expected when the experimental difficulties are considered, and it may be quite well assumed that for practical purposes, the law $c^n t = \text{constant}$, is true, since it agrees within experimental error with results obtained by the best methods at present known.

It will be seen that in the above tables, n is assigned the following values: 5·5 for phenol, 3·8 for mercuric chloride, and 0·86 for silver nitrate. In all cases the bacteria disinfected are *B. paratyphosus.* The figure for disinfectant 'A' is probably about 8·5, but measurements with this substance are very difficult, and the present experimental data insufficient for more accurate deductions. These numbers are probably constant only at a constant temperature, and may vary with the bacteria disinfected, though the figures for phenol in the cases of *B. paratyphosus* and *Staph. pyogenes aureus* are the same. Information on this point would be interesting, but unfortunately is not at present available. However, from the results already obtained, it does seem possible to derive a little insight into the process of disinfection, and perhaps the following idea is not wholly unreasonable.

Each bacterium is composed of a number of molecular groups, and some or all of these contain a chemical compound which can react chemically with, say, N molecules of poison. When disinfection begins, this substance will start to react. Now, although it is capable of reacting with N molecules of poison altogether, it is evident that the substance, which we may denote briefly by the symbol X, will react initially with a smaller number of molecules and thereby form various compounds. Thus, after a short period of disinfection, there will exist in the bacterium a certain number of molecules of X which have not reacted at all, some molecules of X combined with one molecule of poison, some of X combined with two molecules of poison, and so on up to N. We must suppose however that when X has combined with more than a certain definite number of molecules of poison, it becomes incapable of performing its original function in the bacterium. Thus, for example, the compound $X + 3$ molecules of poison, may be able to play its part in the life processes of the bacterium, while the compound $X + 4$ molecules of poison may not; or perhaps the union of X with only one molecule of poison may be sufficient to destroy its power of reproduction (the criterion of vitality determined in the experiments). In any case, however, the result is the same. Disinfection will proceed gradually until a certain number of molecules, and hence of molecular groups, are, so to speak, incapacitated, and when this number reaches some definite percentage of the total number existing in the bacterium, the whole bacterium will lose its vitality, or at any rate it will no longer grow. This occurrence enables us to measure the rate of the reaction, for the death of one bacterium indicates that a certain fixed proportion of the molecules of the substance X which it contains has reacted to a definite degree.

The question which now arises is—What is the actual meaning of n as measured above? It has already been mentioned that if the reaction under discussion were an ordinary complete chemical reaction, n would represent the number of molecules of one substance acting with one molecule of another, but the present case is rather different. For instance, suppose the bacterium ceases to grow when 70 °/₀ of the molecules of the substance X which it contains have each combined with at least two molecules of poison. When death occurs, there will be 30 °/₀ of molecules uncombined or in combination with only one molecule of poison, while 70 °/₀ will be combined with two or more molecules, and consequently the reaction is not completed at this stage, although it is the time at which n is measured. Consequently n does not in this case represent the total number of molecules of poison which can combine with one molecule of X, but the average number which have combined when the bacteria become incapable of further growth.

In this connection it is interesting to note the high value of n. The 'order of the reaction' is at least the integer next greater than $n + 1$, and so in the case of phenol we have a reaction of the seventh order at least. This result may at first sight seem rather extraordinary, especially when it is remembered that the ordinary chemical reactions which have so far been worked out, are rarely of an order greater than the third, but it must be remembered that in the case of bacterial proteins we are dealing with substances of exceedingly high molecular weight, and there seems to be no reason why such molecules should not react with a great many molecules of another less complex substance.

In the whole of the above it has been assumed that there is only one kind of active substance in the bacterium, but, of course, all that has been said applies equally well however many such substances exist, and unfortunately no clue is to be obtained by this method as to their number or nature.

Finally, a word may be said with regard to practical applications of the formula given above. These may perhaps be best illustrated by two examples.

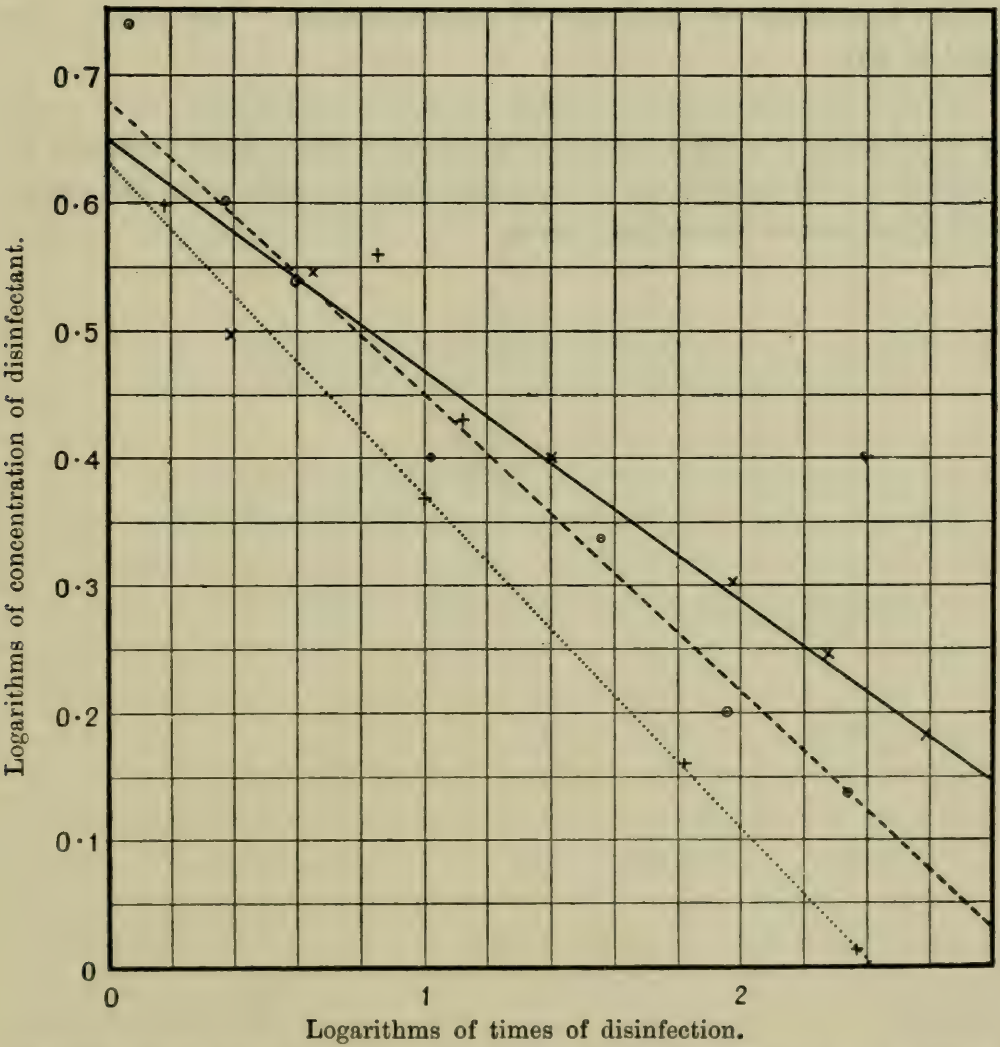
1. A solution of phenol containing 10 parts per 1000, disinfects a culture of *B. paratyphosus* in 25 minutes, another solution takes 35 minutes. What is the strength of the second solution?

Let the strength of the second solution be x.

$$\text{For phenol } n = 5{\cdot}5,$$

therefore, $$5{\cdot}5 \log 10 + \log 25 = \text{const.} = 5{\cdot}5 \log x + \log 35.$$

$$\text{From this } x = 9{\cdot}4.$$



The above figure shows graphically the results contained in Tables II, IV, and V, the corresponding points being denoted by the signs ×, + and 0 respectively. The straight line drawn in full represents the variations in the case of phenol, the dotted line those of mercuric chloride, and the broken line those of silver nitrate. In order to represent all three lines on the same diagram a constant quantity has been added to or subtracted from some of the values of the logarithms as deduced from the tables. This, of course, does not alter the slope of the lines. In the case of silver nitrate the vertical scale is only one fifth of that in the other two cases, and therefore the true slope is five times as steep as it appears to be.

2. A disinfectant takes 9 minutes to disinfect a given culture when the concentration is 7 parts per 10,000 and 50 minutes when the concentration is 5 parts per 10,000. What concentration would disinfect in 15 minutes?

This is most simply solved graphically. Logarithms of the quantities given are taken and marked on a diagram where concentration is plotted against time. The two points thus obtained are joined by a straight line, and the point where this cuts the line representing time 15 minutes, shows the logarithm of the required concentration. The result thus obtained is 5·7.

In conclusion I should like to express my most hearty thanks to Miss Chick for allowing me to use her results, and also for much detailed information concerning them.

On the Occurrence of Nuclear Dimorphism in a Halteridium parasitic in the Chaffinch, and the probable connection of this parasite with a Trypanosome.

By

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In the course of an investigation on the Hæmatozoa of birds, undertaken as Mackinnon Student, I have recently observed certain forms which are of great importance in reference to Schaudinn's view of the ontogenetic relationship between a Trypanosome and a Halteridium of the "Little Owl."

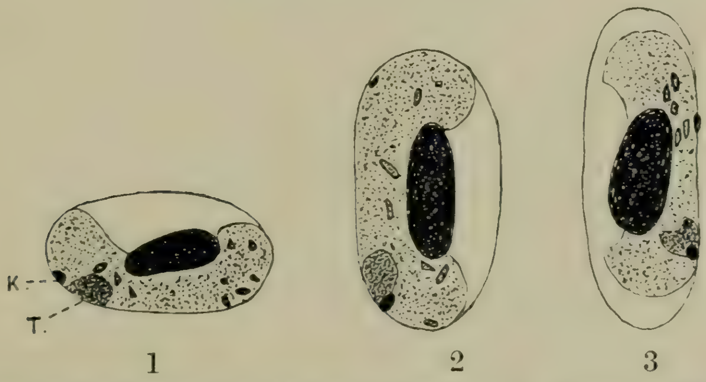
The material was furnished by a chaffinch, known to be infected with a Trypanosome, which was found to be heavily infected with Halteridium also, towards the end of last June. The preparations of which I shall take account in this note were all made between 1 and 3 a.m.; they comprise smears from the peripheral blood, the heart-blood, and from most of the organs. Unfortunately the lungs were forgotten—an omission which I have deeply regretted. The smears were all well fixed with osmic vapour and stained by some modification of the Romanowsky method. Some of the preparations of the peripheral blood were left for a few minutes before smearing, with or without the addition of a drop of salt-citrate solution. As a result, in these smears free fully-formed microgametes and free rounded-off female elements can be more or less readily found.

The Halteridia being so numerous in this bird at that time, I confess I expected, from Schaudinn's account, to have little or no difficulty in finding various stages in the transition of the parasites from an active, trypaniform phase, to a resting, intra-cellular, Halteridium-phase and vice-versâ —always supposing, that is, such a connection exists in this case. A general examination of the more likely slides, including smears from the bone-marrow, etc., showed no indications of such behaviour. Trypanosomes of any kind were very scarce as compared with the great number of Halteridia present, and the few individuals noticed were manifestly larger than the largest Halteridia. I was unable to do more than make a somewhat cursory examination at the time, as I was very much occupied with the research in another direction; and owing to the pressure of other work subsequently, it was not until autumn that I was in a position to begin the laborious and time-consuming process of systematically searching these slides. I am pleased to say this study has now yielded me some most interesting results, and further search would yield, I believe, more yet. As, however, I shall probably have to leave the work at this point for some time, I think it worth while to publish this note. I propose to state shortly those observations made up to the present which bear upon the above question, and to consider briefly the meaning which, it seems to me, is to be attached to them.

Fully-grown Halteridia of three types occur, male and female forms with the usual well-known distinguishing features, and a third type corresponding to the "indifferent," or non-sexual form of Schaudinn; the last-named type is distinguished from a female form by its much lighter staining cytoplasm, and from a male form by its compact, denser nucleus. This indifferent type is by far the least common of the three in these slides. Halteridia of all sizes, however, are to be found in the red blood-corpuscles, from very minute, oval or pear-shaped forms, 2 μ or less in diameter, up to the large adult individuals. Many different phases can often be seen in a few fields of the oil-immersion lens, particularly in liver-smears. A very large number of Halteridia have passed under my eye, but I have never seen the least sign of endogenous multiplication (schizogony) in any of the adult individuals in the red blood-corpuscles, i. e. never anything approaching the nuclear fragmentation and segmentation of the cytoplasm at the two ends, which was described by Labbé.[1] The minute forms do not arise, I am convinced, by the division of the large ones.

Many of the Halteridia exhibit in regard to their nuclear structure a condition which undoubtedly represents nuclear dimorphism. By the term "nuclear dimorphism" is understood a characteristic separation of the nuclear material into two constituents, a larger body staining red with Romanowsky modifications, and a smaller one, which is much denser and stains much darker. These two nuclei, which I have distinguished[2] as tropho- and kineto-nucleus respectively, are of regular occurrence in Trypanosomes and other parasitic

TEXT-FIGS. 1–3.



Female individuals of Halteridium. 1. From peripheral blood, left a couple of minutes before smearing. 2. From liver-blood; and 3, from peripheral blood (of living bird), both smeared at once. *K.* Kinetonucleus, or kinetonuclear element. *T.* Trophonucleus, or trophonuclear element. Fig. 1 × 2000; Figs. 2 and 3, × 2500.

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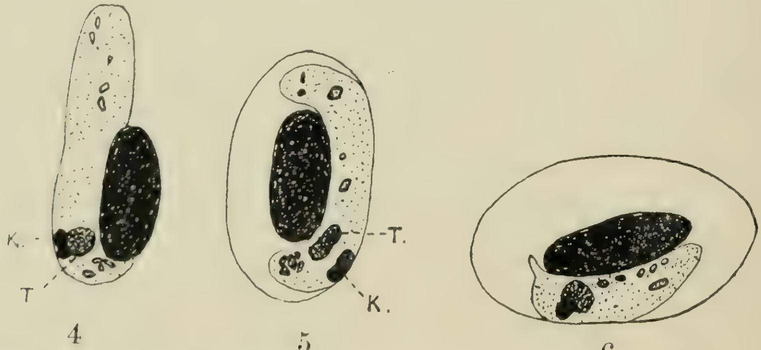
[1] 'Arch. Zool. Exp.,' ser. 3, vol. 2, p. 55, 1894.

[2] "The Hæmoflagellates," 'Quart. Journ. Micr. Sci.,' vol. 50, p. 151, 1906.

Flagellates. I first observed this condition in some of the large female individuals, in which it appears as seen in text-figs. 1–3. Lying close to the ordinary nucleus, generally in contact with it, is a much smaller body, which stains much more deeply than the large one, at times appearing almost black (*K.*). This little body is generally ovoid or round, but in some cases tends to have a rod-like shape. There is no possibility of confusing this nuclear body with a large pigment-grain, or a collection of grains. It lies usually near to the outer surface of the body. In parasites of the indifferent type this nuclear element, which I homologise with a kinetonucleus, is larger and may approximate to the size of the other nucleus (trophonucleus). It may be round (text-fig. 6) or, frequently, it is more or less dumb-bell-shaped (text-fig. 5), as if it were composed of two halves. It is important to note that this nuclear dimorphism can be readily recognised in many of the minute forms, the kinetonucleus being a small, deeply-staining grain lying close to the ordinary nucleus, generally at one side (text-fig. 12). This feature gives several of these small individuals a striking resemblance to the resting-phases described of various Herpetomonadine parasites.

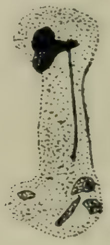
TEXT-FIGS. 4–6.



Indifferent forms of Halteridium. 4 and 5. From heart blood, smeared straightway. 6. From peripheral blood, after addition of a drop of salt-citrate solution, and interval of a couple of minutes before smearing. *K.* Kinetonucleus, or kinetonuclear element. *T.* Trophonucleus, or trophonuclear element. × 2500.

In none of the male forms scrutinised so far have I been able to make out a distinct kinetonuclear body. This may be because it is not differentiated, as a compact organella, from the rest of the diffuse nucleus of this type. (I am inclined to think, however, that it is present in the free, fully-formed male gametes, although it is difficult to feel quite sure; I shall refer again to the structure of these delicate elements.) Moreover, in many of the female individuals, the kinetonuclear element is by no means so prominent or separate as in the examples figured, which have been chosen to show this feature as clearly as I have observed it. Others, again, do not show it at all. It is quite probable that at times the two constituents are incorporated in one nucleus, as was said, indeed, by Schaudinn to occur at certain periods.

Text-fig. 7.



Free form, from heart-blood; smear made at once. × 2500. (Probably the upper nuclear body is the kinetonucleus.)

It is highly significant, I think, that up to the present, when an intra-cellular parasite has been known to exhibit nuclear dimorphism, although lacking in that stage any flagellum or obvious sign of Flagellate affinity, it has been subsequently found to be really a phase in the life-cycle of some Flagellate form; in other words, the knowledge of nuclear dimorphism in a parasite has hitherto heralded, as it were, the discovery of its intimate connection with a Flagellate. The classic instance is the Leishman-Donovan body, whose flagellar phase was first made known by Rogers. Again, Schaudinn himself maintained that certain Piroplasmata showed this character, and others (e.g. Lühe) have since corroborated him. Only recently Miyajima[1] has carried the matter a step further, and obtained the development of unmistakable Trypanosome phases in cultures from a Piroplasma of cattle in Japan. Hence, the occurrence of this feature in Halteridium even regarded by itself is, to my mind, most suggestive; and it is with very great pleasure that I bring forward what is, I believe, the first definite piece of evidence tending to confirm one, at all events, of Schaudinn's celebrated conclusions.

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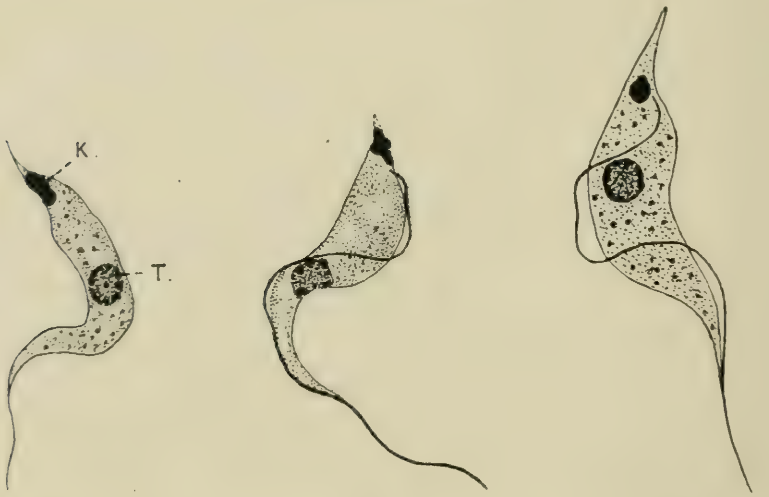
[1] 'Philippine Journ. Sci.,' ser. B, vol 2, p. 83, 1907.

Text-figs. 8–10.



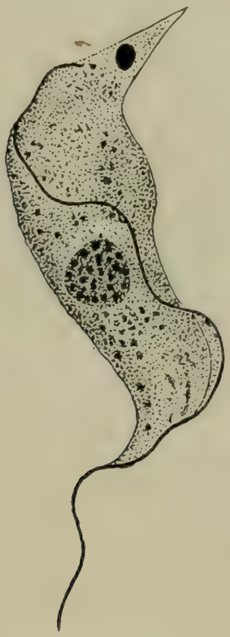
8 9 10

Trypanosomes from a bone-marrow smear. In 8 the flagellum is very faintly stained, and its course along the side of the body cannot be followed. *K.* Kinetonucleus. *T.* Trophonucleus. × 2500.

Stimulated by this discovery, I have striven to find some phases showing the actual passage from a Halteridium-form to a Trypanosome-form, but in the case of the parasites of the chaffinch such phases appear to be very few and far between. This is, unfortunately, only what might be expected from the great scarcity, comparatively, of the Trypanosomes themselves. I have obtained certain indications, which, so far as they go, point to such a transformation, or fit in with it; and I have observed nothing which in any way invalidates this view.

Before leaving the Halteridium side of the question, there is one phase which I have found, to which I attach considerable importance (text-fig. 7). This is in a smear, well fixed and well stained, which was made from heart-blood and smeared straightway. The parasite is not altered or deformed in any way mechanically; I am confident that it represents a normal phase. The individual in question is free in the blood. It is of the indifferent type, and the two nuclei are in close contact, both dense and deeply staining. The pigment grains are all near one end of the cytoplasm, in a position in which they might easily be got rid of. The most interesting point is the presence of an unmistakable thread or line, which is stained bright red. This starts from a short, transverse, deep-staining band, adjoining the two nuclei, and runs down part of the length of the body near to one side, terminating in a definite granule. The narrow portion of the cytoplasm between it and the margin of the body has a distinctly reddish tinge, the rest of the protoplasm being of the usual blue colour. The only explanation I can give of this thread is that it represents the "central spindle"

TEXT-FIG. 11.

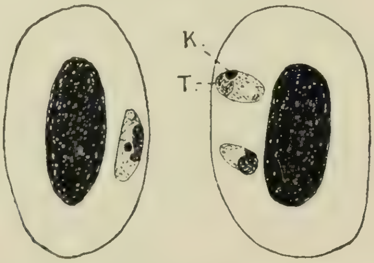


Large Trypanosome, from peripheral blood. × 2500.

described by Schaudinn, which in later development becomes the flagellar border of the membrane, i. e. the proximal part of the flagellum.[1]

Turning to the question of the Trypanosomes, I have now succeeded in finding, in this series of slides, particularly in smears of the bone-marrow, a few very small Trypanosomes, forms which are no larger than the large individuals of Halteridium. When these little Trypanosomes (as in text-figs. 8 and 9) are compared with the large forms in the blood at this time (e. g. fig. 11), the difference in size is seen to be very marked. Between these extremes all intermediate stages can be found. I could figure a series of regular gradations from the one to the other. Now, I have never seen the slightest indications of division; even at this time, when the Trypanosomes are less infrequent than at other times, e. g. early spring or late autumn. Hence, I consider that the larger forms have grown from smaller ones, and not that the small ones have arisen, by successive multiplication, from the large individuals. With regard to the origin of the very small Trypanosomes themselves, I am certainly disposed to think that they arise from adult Halteridia. This seems to me to be the most reasonable conclusion to arrive at, having regard to the data I have ascertained so far.

TEXT-FIG. 12.



Small forms of Halteridium, from a liver-smear. The right-hand figure shows the appearance when two parasites are in one corpuscle, which is of frequent occurrence. *K.* Kinetonucleus, or kinetonuclear element. *T.* Trophonucleus, or trophonuclear element. × 2500.

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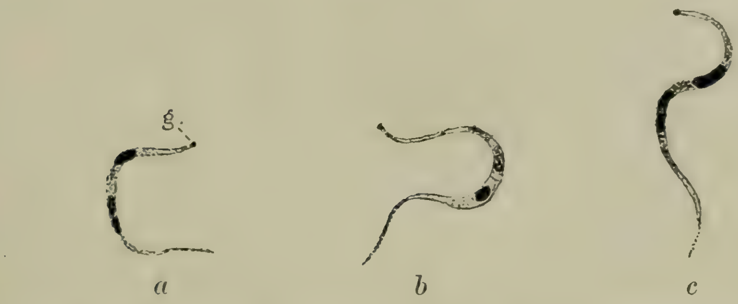
[1] I have now found this phase in two or three instances.

It is true that the number of Trypanosomes is very small in proportion to that of the Halteridia, but the disproportion is largely reduced if the sexual forms of the intracellular parasites are left out of account; and, as I have mentioned, the great majority of the Halteridia are of the male or female type. It is probable that the Trypanosomes are reinforced in number from the indifferent Halteridia as a rule; and from the female forms only in exceptional circumstances, e. g. perhaps late in the season, if unable to become fertilised. I do not think it likely that the male individuals give rise to ordinary Trypanosomes at all.

In some of my preparations very good examples of microgametes are to be found. These had, in life, freed themselves from the residual body of the gametocyte, were actively motile, and for all I know to the contrary were fully-developed male elements. They have been examined under the best obtainable conditions. This is essential, since the width of these fine organisms is only from ·5 to ·7 μ. Unfortunately, too, the gamete takes up the stain so intensely, that it is often difficult to differentiate sharply between the nuclear and cytoplasmic portions. The chief evidence of trypaniform structure which I hoped to obtain was, of course, the presence of an undulating membrane, as shown by Schaudinn in his schematic figure. I examined, particularly, deeply stained specimens, thinking the flagellar border, standing out from

Text-fig. 13.

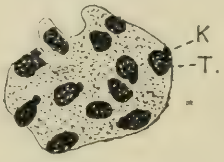


Male gametes, from peripheral blood, left a couple of minutes before smearing. *b* is the least intensely stained, *c* the most so. *g*. Centrosomic granule. × 2500.

the body, would be more distinguishable in such. I was not able, however, to satisfy myself of the existence of such an organella. I am uncertain whether there is one or not; it may be that I have not been able to discern it owing to its contiguity to the general cytoplasm.

In certain respects the structure of these gametes does undoubtedly agree with that described by Schaudinn. At one end, which is abruptly rounded, there is an unmistakable granule, which stains more deeply than the neighbouring cytoplasm (text-fig. 13, *g.*). This organella is apparent in most of the individuals observed, and most probably corresponds to the anterior centrosome of Schaudinn. The opposite end is finely tapering, and comparable to the cytoplasmic tail. I have not been able to make out any elaborate nuclear details, but I am inclined to think a kinetonuclear body can be distinguished; at all events, one of the chromatic masses, of which there appear to be three or four, is usually larger and more deeply-staining than the others (cf. fig. 13).

TEXT-FIG. 14.



Dividing parasite, probably giving rise to small Halteridia; from the bone-marrow. *K.* Kinetonucleus, or kinetonuclear element. *T.* Trophonucleus, or trophonuclear element. × 2500.

In conclusion, I have a few words to add with regard to the origin of the minute intra-cellular Halteridia. Two or three weeks after this series of preparations was made, I saw for the first time Aragao's account[1] of his work on the Halteridium of the pigeon. Aragao has found that the very young forms, which enter the red blood-corpuscles, originate by the multiple division (schizogony) of large parasites which occur in the endothelial cells of the lung-capillaries. This worker thinks that the form or phase

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[1] 'Arch. Protistenk.,' 12, p. 154, 1908.

—whatever it may be—in which the Halteridial parasite enters the blood from the Invertebrate host, in this case a Lynchia, passes first into one of these endothelial cells. In this position it grows and subsequently gives rise to a progeny of little individuals, which, when set free, penetrate the red corpuscles and become the well-known Halteridia.

I have no doubt a similar process occurs in the case of the parasites of the chaffinch. Most unfortunately I omitted to make smears from the lungs. In a smear from the bone-marrow, however, I have come across two instances of a phase which, I believe, corresponds to the segmenting forms described by Aragao. One of these parasites is drawn in text-fig. 14. It is free, having evidently broken loose from the cell, perhaps a leucocyte, in which it was parasitic. It is in process of multiple division, possessing several nuclei. The most interesting point to notice is that some of these nuclei show nuclear dimorphism. Associated with the larger, more obvious nucleus is a small, deeply-staining grain, which probably represents the kinetonuclear element. Two or three of these "double" nuclei closely resemble the double nuclei of the minute Halteridia in the red blood-corpuscles (cf. text-figs. 12 and 14). Unfortunately, the specimen is rather darkly stained, and I cannot be sure of this feature in all the daughter-nuclei.

The connection of Halteridium with a parasite of cells other than red corpuscles (endothelial cells, leucocytes, etc.), made known by Aragao, furnishes another link in the complicated chain of events, which, according to all indications, make up the complete life-cycle of this form. It is instructive to note that Leishmania donovani, which is now admitted by everyone to be the intra-cellular phase of a Flagellate, is a parasite of such cells; and it is not yet certain whether it invades the red corpuscles. Halteridium is probably a stage in the life-history of a Trypanosome, which has advanced a step further and become adapted also to the red corpuscles.

THE LISTER INSTITUTE,
December 10th, 1908.

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